ORGANIC ACID EXUDATION AS A MECHANISM OF ALUMINUM-TOLERANCE IN TARO [*Colocasia esculenta* (L.) Schott]

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

AGRONOMY AND SOIL SCIENCE

DECEMBER 1997

By

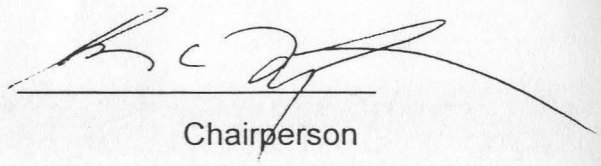
Zhong Ma

Thesis Committee

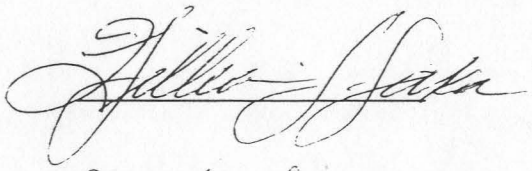
Susan Miyasaka, Chairperson
William Sakai
Chung-shih Tang
Michael Tanabe

We certify that we have read this thesis and that, in our opinion, it is satisfactory in scope and quality as a thesis for the degree of Master of Science in Agronomy and Soil Science.

THESIS COMMITTEE



Chairperson



ACKNOWLEDGMENTS

I would like to thank my major adviser, Dr. Susan C. Miyasaka, for her constant guidance and support which made my graduate research completed as planned. Two years after I started working with her, I came out realizing that I had many opportunities of valuable training, which would definitely be an asset in my future academic pursuit. My thanks to my committee members, Dr. Tanabe, Dr. Sakai, and Dr. Tang, for their many insightful suggestions and intellectually stimulating conversations. I enjoyed those talks greatly.

I would also like to thank all the nice people in our neighbor labs in Hilo, and professors, secretaries, and fellow graduate students in the department at Manoa campus, for their help in solving the seemingly trivial problems I had, and for the fun we had together.

My thanks go to all these wonderful people. You are lasting memories.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Introduction

Aluminum Phytotoxicity: A Major Problem in Acid Soils

Aluminum (Al) toxicity, associated with soil acidity, is a major factor limiting plant growth in many regions of the world (Kamprath, 1984). Soils in the humid tropics have developed under conditions that favor the formation of acid soils. Approximately two-thirds of the soils in the humid and sub-humid regions are highly weathered and likely to have acidity problems (Kamprath, 1984). Soil acidity decreases plant growth through deficiencies of phosphorus, molybdenum, or calcium, and/or toxicities of Al, manganese, and hydrogen ions (Ritchie, 1989). In particular, Al toxicity has been recognized as one of the most common causes of reduced yields (Foy, 1984).

The usual method to correct soil acidity is through liming the soil to a pH of 5.5 to 6.0. However, the quantities of lime needed are not always available at economical prices, particularly for farmers in developing countries (Foy et al., 1987). More importantly, even when surface soils are limed, excess soluble or exchangeable Al in acid subsoils may restrict root development and interfere with the use of subsoil water and nutrients (Foy et al., 1987). In most soils, mixing limestone with the plow layer does not effectively neutralize subsoil acidity (Wright et al., 1985), and liming subsoils is generally not economically feasible (Foy et al., 1987).

An alternative or supplemental approach is to grow plants that have greater tolerances to Al. The magnitude of genotypic differences in sensitivity to acid soil problems holds particular promise in overcoming the limitations to plant growth brought about by soil acidity (Foy, 1984). Efforts to select and breed such plants are being made worldwide, especially in developing countries that depend on a low input system of agriculture (Dambroth and Bassam, 1982).

Although plant species and cultivars within species differ widely in their capacity to withstand Al stress, the physiological or biochemical mechanisms involved have not been determined (Foy, 1984). Knowledge of such mechanisms would be valuable in breeding for plants that fit problem soils (Foy et al., 1987). Currently, hypotheses on Al resistance are categorized into the external resistance mechanisms (exclusion mechanisms), which serve to limit the rate of entry of Al into the cytosol, and the internal resistance mechanisms, which operate within the symplasm (Taylor, 1991).

The objectives of this study are:

a. To determine whether the release of oxalic acid and/or other organic acids is observed under Al stress, and, if organic acids are released, how the release varies with time.

b. If organic acids are released in response to Al stress, then to determine the effect of Al on the exudation of organic acids from the roots of taro grown under Al-stress in aseptic conditions.

c. To test whether the release of organic acids is specifically induced by Al or a response to low phosphorus, or both.

d. To examine the ameliorative effects of the exudated organic acids.

e. To determine the existence of differential Al-tolerance within seven tissue-cultured taro cultivars.

Chapter 2

Literature Review

Aluminum Phytotoxicity in Solutions and Soluble Aluminum Species in Soils

Aluminum can be toxic to plants in acid soils with pH values of or below 4.5, the condition under which representative values of Al concentrations are between 1 and 30 mg L^{-1} (37.06 and 1111.93 μM) (Black, 1957). The susceptibility to Al toxicity differs considerably among and within plant species (Haug, 1984). For example, in maize (*Zea mays L.*) cultivars grown in dilute, simple salt solutions ($CaCl_2$, Ca^{2+} activity=200 μM; pH 4.3), low Al^{3+} activities (6-9 μM) had little effect on root growth over 6 to 48h in the Al-tolerant cultivar (Pellet et al., 1995). The same Al activities elicited a significant inhibition of root growth in the Al-sensitive genotypes (Pellet et al., 1995). A similar differential Al tolerance was also observed for a tolerant ('SA3') and a sensitive (*'Tuxpeno'*) maize cultivars both in full nutrient solutions and in the field (Kasim and Wassom, 1990; Kasim et al., 1990; as reported by Pellet et al., 1995). In snapbeans (*Phaseolus vulgaris L.*), the roots of an Al-tolerant cultivar 'Dade' were able to survive and grow in nutrient solutions containing 148 μM Al, whereas the roots of an Al-sensitive cultivar 'Romano' died as soon as they contacted solutions with the same Al concentration (Miyasaka et al., 1991). At 74 μM Al, the roots of 'Romano' were slightly stunted and exhibited the "coralloid" roots typical of Al toxicity (Miyasaka et al., 1991). In wheat (*Triticum aestivum L.*), upon exposure to 100 and 200 μM Al in solution, root elongation in Al-sensitive

cultivars ('Roblin' and 'Katepwa') was reduced by 30% and 65%, respectively, whereas root elongation in resistant cultivars ('Atlas 66' and 'Maringa') was reduced by only 15% and 30% (Basu et al., 1994b). In taro, a solution level of Al at 1330 μM elicited the greatest difference in root growth inhibition among four taro cultivars differing in Al-tolerance (Calisay, 1996). In another study (Miyasaka et al., 1993a), an initial solution Al level of 890 μM was found to result in the greatest separation of growth differences between taro cv. Lehua Maoli and Bun long in their response to Al (Miyasaka et al., 1993a). Compared to many temperate crop species (e.g., wheat, snapbeans), taro is relatively tolerant of Al as a tropical species, and is able to tolerate Al levels of up to 440 μM Al with little reduction in growth (Miyasaka et al., 1993a).

Soluble Al species in soil solution may be broadly divided into two groups (Ritchie, 1989): 1) monomers (e.g., Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^+$, $Al(OH)_4^-$, $Al(SO_4)^+$, $Al(SO4)_2^-$, AlF^{2+}, AlF_2^+, AlF_3^0, or AlF_4^-); and 2) polymers (e.g., Al_2, Al_3, Al_{13} polymers with hydroxyl ions, phosphate or silicate ions) (Ritchie, 1989). Part of the difficulty of studying Al-related process in plants can be attributed to the complex chemistry of Al (Kinraide, 1991).

Aluminum hydrolyzes in solution. At pH levels below 4.5, Al^{3+} dominates, whereas $AlOH^{2+}$ and $Al(OH)_2^+$ species form as pH increases (Delhaize and Ryan, 1995). At a near-neutral pH, the solid phase $Al(OH)_3$ occurs, whereas $Al(OH)_4^-$ (aluminate) dominates in alkaline conditions (Delhaize and Ryan, 1995). As mentioned before, monomeric Al cations can bind to various organic and inorganic

ligands such as PO_4^{3-}, SO_4^{2-}, F^-, organic acids, proteins, and lipids (Delhaize and Ryan, 1995).

The relative toxicities of the many ionic Al species are still not completely clear. However, there is evidence suggesting that Al^{3+} and/or the monomeric Al-hydroxy species are the forms of Al that are the most correlated with depressed yields of several plant species (Blamey et al., 1983; Cameron et al., 1986). Wright et al. (1987) reported that estimates of concentrations of monomeric Al species were significantly related to root and shoot growth of subterraneum clover (*Trifolium subterraneum L.*) and switchgrass (*Panicum virgatum L.*). Pavan et al. (1982) found that the relative root growth of coffee seedlings in four acid soils was highly correlated with the activity of free Al in solution. Because the activities of monomeric Al-hydroxy species are interrelated, it is difficult to identify the actual toxic species. For this reason, use of the sum of the activities of monomeric Al species in soils or solutions as the putative toxic species seems most warranted (Taylor,1987). However, Kinraide (1991) proposed that the apparent toxicity of mononuclear hydroxy-Al to dicotyledonous plants is very problematical because of the possible confounding effects from polymeric Al_{13}, and concluded that Al^{3+} and Al_{13} are both rhizotoxic (Kinraide, 1991). Although polymeric Al (Al_{13}) was reported to be quite toxic (Parker and Bertsch, 1992), its natural occurrence and contribution to soil toxicity are unknown.

Uptake, Movement and Distribution of Aluminum in Plants

In a study comparing kinetics of Al uptake between Al-tolerant and Al-sensitive cultivars of the same species, Zhang and Taylor (1989, 1990) demonstrated that there existed a two-phase uptake of Al by excised roots of both tolerant and sensitive wheat cultivars, i.e., a rapid phase of uptake in the first 30 minutes, followed by a linear phase of uptake up to 3 hours. The rapid stage of the dual kinetics of Al uptake was interpreted as representing movement into the apoplasm, and citric acid was shown to be the most effective in desorbing the loosely bound Al from the putative apoplasmic compartment. The linear stage was considered to include metabolism-dependent adsorption of Al on cell walls and absorption across the plasma membrane into the symplasm. No desorption was observed from this linear phase.

There exist two transport pathways of ions through cortical cells in roots, namely, symplasmic and apoplasmic pathways. Polyvalent cations such as Al ions have been considered to pass through the cell wall via the apoplasmic pathway (Wagatsuma, 1984). However, evidence of Al accumulating in nuclei (Matsumoto, 1988) showed that some Al ions could also use the symplasmic pathway. Since the plasma membrane constitutes a barrier to Al entry due to the insolubility of Al ions in lipid bilayers, Al ions probably cross the plasma membrane as a neutral Al ligand through membrane-bound proteins, by endocytosis, or via stress-related lesions (Delhaize and Ryan, 1995). The development of the Casparian strip and the formation of suberin lamella in endodermal cell walls constitute a barrier in the

apoplasmic transport of Al (Wagatsuma, 1984). Therefore, it is assumed that Al enters the xylem vessel either prior to suberization, or by the disruption of the membrane's lipid bilayer due to considerable accumulation of Al on the surface of cortical and endodermal cell membranes (Wagatsuma, 1984). Aluminum could also enter into roots via disruption of the functional apoplasmic barrier at the endodermal or exodermal Casparian band due to the emergence of lateral roots (Peterson, 1988).

The aluminon staining method was used to localize absorbed Al in different longitudinal portions of pea (*Pisum sativum L.* 'Kinuzaya') after treatment with 20 mg L^{-1} Al at pH 4.2 for 12 to 48 h (Wagatsuma, 1984). At the root tip, Al was mostly accumulated in the epidermis, hypodermis, cortex, and endodermis adjacent to immature xylem, and only a small amount of Al was accumulated in immature xylem. At proximal root sections, Al accumulation occurred mostly in the epidermis, hypodermis, outer cortex, and endodermis adjacent to the xylem, and a little Al was accumulated in the stele. At a younger stage before complete suberization of endodermal cells, Al accumulation in the stele was observed both at the endodermal and the stelar parenchyma cells adjacent to protoxylem; whereas, after complete development of the xylem and endodermis, this accumulation of Al occurred mainly at the endodermal cells adjacent to protoxylem (Wagatsuma, 1984). In another study of early entry of Al into cells of intact, Al-sensitive soybean roots (Lazof et al., 1996), it was found that the majority of the Al accumulated in

cortical cells of the root-tip, intermediately developed, and mature regions of the root during an initial 4 h exposure to 38 μM [Al^{3+}].

Aluminum-accumulator plants, such as tea (*Camellia sinensis*), could contain as high as 30,690 mg kg^{-1} Al in old leaves as compared with 262 mg kg^{-1} Al in young leaves (Matsumoto et al., 1976). The Al content of non-accumulator plant tops is generally in the range of tens to hundreds of mg per kg of dry matter and is considerably lower than that in roots (Wagatsuma, 1984). Differences among plant species in translocation of Al to tops have been recognized (Wagatsuma, 1984).

Aluminum Toxicity in Plants

In many crop plants the first observable symptom of Al toxicity is a reduction in root elongation (Foy, 1984). The affected roots are stubby and thick (Clarkson, 1981).

The location of the primary injury to roots is disputed. Ryan et al. (1993) reported that Al applied to the root tips (terminal 2.0-3.0 mm of root) of an Al-sensitive maize (*Zea mays L.*) cultivar inhibited root growth; whereas Al applied to the elongating zone (3.0 mm proximal to terminal zone) of the roots had no significant effect on root growth. However, Wagatsuma et al. (1987) showed that damage due to Al on roots of barley, oats, rice, maize and peas was not restricted to the meristematic region and suggested that the observed damage in more proximal cells behind the root tip was associated with damage to the plasmalemma of epidermal and cortical cells.

The physiological and biochemical bases of Al phytotoxicity are still not clear (Taylor, 1987). Aluminum appears to have several primary toxic lesions at the membrane level, in the cytosol, and possibly in the apoplasm. Possible mechanisms by which Al may disrupt cellular function include: (1) disruption of plasma membrane structure and function; (2) inhibition of DNA synthesis and mitosis; (3) inhibition of cell elongation; and (4) disruption of mineral nutrition and metabolism (Taylor, 1987; Kochian, 1995).

The toxic effect of Al on membrane integrity and function was shown in the microorganism, *Thermoplasma acidophilum*, by Vierstra and Haug (1978), who found that membrane lipid fluidity was reduced by Al stress. Akeson et al. (1989) reported that Al^{3+} had a 560-fold higher affinity for the phosphatidylcholine surface than Ca^{2+}, thus reducing adsorption and uptake of cations (notably Ca^{2+}). He suggested that this adsorption of Al^{3+} could contribute to Al uptake into the cytoplasm by endocytosis (Akeson et al., 1989).

Upon entering the symplasm, the prevailing pH of 6.5-7.0 and the abundance of potential ligands will maintain the concentration of Al^{3+} ions at a very low level (Delhaize and Ryan, 1995). The neutral ion $Al(OH)_3.3H_2O$ limits the solubility of free $Al^{3+}.6H_2O$ to less than 10^{-10}M at pH 7.0 (Taylor, 1987). In addition, the presence of phosphate in the cytosol reduces the solubility of $Al^{3+}.6H_2O$ by precipitation of $Al(OH)_2H_2PO4$ (Taylor, 1987). However, due to the high binding affinities of Al for many metabolically important molecules, such as inorganic phosphate, nucleotides, DNA, proteins, carboxylic acids, phospholipids, etc. (Haug, 1984), even a very low

concentration of Al in the symplasm is by no means non-phytotoxic (Taylor, 1987). Since the plasma membrane and nuclear envelope are both biological membranes that have a number of basic properties in common (Hall et al., 1974b), it is possible that Al could cross the nuclear envelope as some form of an Al-ligand complex, in a similar manner by which it crosses the plasma membrane, and reaches the nucleus for a phytotoxic effect on DNA or mitosis.

That Al could disrupt normal functioning of the nucleus was demonstrated by Matsumoto (1988). He found that Al absorbed by the roots of peas (*Pisum sativum* ' Alaska') was related to alteration of the chromatin structure. Matsumoto (1988) suggested that the condensation and stabilization of chromatin structure due to the binding of Al to DNA could cause reduced template activity, thereby inhibiting cell division at root tips.

However, Sampson et al. (1965) found that DNA replication still occurred in roots of barley (*Hordeum vulgare*) after 48h of exposure to Al, while the number of cells in mitosis was reduced to zero after 24h of exposure. In addition, Wallace and Anderson (1984) found that Al-induced inhibition of root elongation in wheat preceded measurable inhibition of DNA replication by at least 2h. Thus, Al interaction with DNA may not be a primary lesion in all species. It may well be that toxic lesions have occurred at other sites prior to damage to DNA, and disruption of other metabolic processes may also contribute to the inhibition of cell division.

In addition to inhibition of cell division, Al could also inhibit cell elongation. A reduction in cell elongation could be caused by its toxic effect on cell wall

components and/or an Al-induced reduction in the rate of cell wall synthesis (Taylor, 1987). Blamey et al. (1990) proposed that Al could precipitate pectins in cell walls, thus decreasing the protective capacity of pectins and enabling further expression of Al toxicity. Thirty to forty percent of Al taken up by wheat roots was reported to be associated with cell walls during a 48h Al-exposure (Tice et al., 1992). A toxic effect of Al on Golgi secretory function in corn (*Zea mays L.*) roots was found by Bennet et al. (1987). They observed that the damage caused by Al was primarily directed at the Golgi apparatus in peripheral cap cells after a 29h-Al treatment, at which point no discernible changes in nuclear structure were found. They noticed that changes in the Golgi apparatus activity coincided with a decrease in frequency of which Golgi apparatus-derived material was accumulated between the plasmalemma and cell wall. The cell wall is regarded to form from the extracellular deposition of polysaccharide and protein. There is evidence that Golgi bodies and associated membranes are sites of polysaccharide biosynthesis and are involved in the secretion of polysaccharide material into the cell wall (Hall et al., 1974a). Clearly, a reduction in the rate of the synthesis of cell wall component material will lead to a decrease in cell elongation.

Finally, broad disruptions in patterns of mineral accumulation have been reported to occur in Al-stressed plants (Taylor, 1987). However, the hypothesis that toxic effects of Al are a result of Al-induced mineral deficiencies is disputable, since rapid toxic effects of Al have been observed in many cases. However, although Al-induced mineral deficiencies are not likely to be direct stress injuries, secondary

stress injuries are also very important. For example, similarity of some Al toxicity symptoms and Ca deficiency symptoms has been observed (Foy et al., 1967). In addition, rapid toxic effects could result from effects on mineral ion metabolism which are not directly related to mineral deficiency (Taylor, 1987). For example, Haug (1984) found that Al ions in vitro lead to profound structural alterations of calmodulin, a Ca-dependent regulatory protein required for a wide variety of cellular functions.

Physiological Mechanisms of Aluminum-tolerance

As discussed earlier, although plant species and cultivars within species differ widely in tolerance to Al, the specific physiological or biochemical mechanisms involved in differential Al tolerance have not been clearly determined. Since it is not known whether the primary Al lesion is extracellular or intracellular (Kinraide, 1988), there are many hypothetical mechanisms for Al toxicity. As a result, numerous associated tolerance mechanisms also have been proposed. It is possible that due to varied effects of Al in plants, different species and varieties may have different tolerance mechanisms controlled by more than one gene (Foy et al., 1978). Research on different plants have yielded various conclusions. They can be grouped into two major categories: a) external resistance mechanisms, by which Al is excluded from the symplasm or its adsorption is limited; and b) internal resistance mechanisms, by which Al is detoxified in the symplasm (Foy, 1988; Kochian, 1995; Marschner, 1991; Taylor, 1991).

Within each of the above mentioned two categories, several hypothesized mechanisms have been proposed. Hypotheses regarding external resistance mechanisms include: (1) immobilization at the cell wall or low cell wall cation exchange capacity (CEC), (2) selective permeability of the plasma membrane, (3) formation of a plant-induced pH barrier in the rhizosphere or root apoplasm, (4) exudation of chelate ligands, (5) exudation of phosphate, and (6) Al efflux. Internal resistance mechanisms might also take a variety of forms, including: (1) chelation in the cytosol by organic acids, proteins, or other organic ligands, (2) compartmentation in the vacuole, (3) evolution of Al-tolerant enzymes, and (4) elevated enzyme activity.

It is well established that organic acid-Al complexes are not toxic to plant roots (Bartlett and Riego, 1972; Hue et al., 1986; Kerven et al., 1991). Bartlett and Riego (1972) found that the toxicity of ionic Al^{3+} for maize was prevented by complexing the Al with citrate, EDTA or a soil organic matter extract. According to Hue et al. (1986), detoxification capacities of organic acids correspond with relative positions of OH/COOH groups on the main C chain. In other words, those organic acids favoring the formation of a stable 5 or 6 bond structure with Al form strong chelates with Al, e.g., citric acid. Based on the data obtained by Hue et al. (1986) of the effects of Al on root growth of cotton (*Gossypium hirsutum*) in solution culture, the short-chain, carboxylic acids were classified into three groups: strong detoxifiers (citric, oxalic, tartaric), moderate detoxifiers (malic, malonic, salicylic), and weak detoxifiers (succinic, lactic, formic, acetic, phthalic) (Hue et al., 1986). The role

played by organic acids in plant tolerance to Al has been the subject of considerable speculation (Jones, 1961; Foy et al., 1978; Taylor, 1991). Organic acids have been suggested to play a role both in Al exclusion, via release from the root (Delhaize et al., 1993; Miyasaka et al., 1991), and Al detoxification in the symplasm, where organic acids such as citrate and malate could chelate Al and reduce its toxic effects at the cellular level (Suhayda and Haug, 1986).

Research in Support of an External Resistance Mechanisms Involving Organic Acids:

Plants are known to exude organic acids into the rhizosphere in response to mineral stress. Acetate, aconitate, citrate, glycolate, malate, malonate, oxalate and succinate have been found in root exudates from a number of species (Gardner et al., 1983; Jayman and Sivasubramainam, 1975; Smith, 1976; Vancura and Hovadik, 1965). Evidence has been found to support external resistance mechanisms that involve the chelation and detoxification of Al by organic acids (Delhaize et al., 1993; Basu et al., 1994a; Ryan et al., 1995; Pellet et al., 1996). Much research has been done on wheat concerning the excretion of organic acids in response to Al (Delhaize et al., 1993; Basu et al., 1994a; Ryan et al., 1995; Pellet et al., 1996). Wheat has exhibited up to 10-fold differences in Al tolerance between genotypes. In addition, near-isogenic wheat lines have been developed to differ at a single Al-tolerance locus and they provide simplified systems for the study of Al tolerance mechanisms (Delhaize and Ryan, 1995). Ryan et al. (1995) investigated the relationship between Al tolerance and Al-stimulated malate efflux from root apices of 36 wheat cultivars

differing in Al tolerance. They found a significant correlation between relative tolerance to Al as measured by root growth (relative root length) and the amount of malate released from root apices during a standard Al treatment. These correlations strongly indicated that the Al-stimulated efflux of malate accounted for much of the tolerance exhibited by genotypes under study. Also, growth measurements indicated the amelioration of Al toxicity by exogenous malate. Addition of 20 μM malate to the solution containing 3 μM Al completely alleviated the Al-induced inhibition of root growth.

In support of the above conclusion, Basu et al. (1994a) in their study of wheat root exudation under aseptic conditions found that malate was exuded in higher quantities from roots of Al-tolerant cultivars compared with Al-sensitive ones under control conditions, whereas exposure to 100 μM Al increased exudation of malate from Al-tolerant cultivars by 100-120%, but decreased that of sensitive cultivars. Addition of exogenous malate (250-500 μM) to nutrient media containing 100 μM Al restored root elongation in Al-sensitive cultivars to control levels. Further, they labeled plants with ^{14}C-acetate and found that in tolerant plants, Al increased incorporation of ^{14}C into malate. Malate was the only organic anion radioactively labeled, indicating *de novo* synthesis of malate after exposure to Al, and suggesting a role of malate in Al-resistance.

Delhaize et al. (1993) reported that in near-isogenic wheat (*Triticum aestivum L.*) lines differing in Al tolerance at the Al tolerance locus (Alt1), roots of Al-tolerant genotypes excreted 5 to 10-fold more malic acid than Al-sensitive genotypes at

each time interval over 24h when exposed to 50 μM Al under sterile conditions. Root apices, the principal site of Al injury, were identified to be the primary source of malic acid excretion. Also, their data (Delhaize et al., 1993) indicated the occurrence of continued synthesis of malic acid, and that the excretion was specifically stimulated by Al rather than by low phosphorous. They thus concluded that an Al tolerance mechanism encoded by the Alt1 locus was based on Al-stimulated excretion of malic acid (Delhaize et al., 1993). The relatively high rates of malate efflux stimulated by Al in different wheat cultivars (Ryan et al., 1995; Basu et al., 1994a; Delhaize et al., 1993;) suggested that this response might be a general mechanism for Al tolerance in wheat.

There were also reports of related work done on other crops. In their work on snapbeans (*Paseolus vulgaris L.*), Miyasaka et al. (1991) reported that in sterile culture, an Al-tolerant cultivar released citric acid in a concentration that was 70 times greater when exposed to Al than in the absence of Al, and 10 times greater than those of the Al-sensitive cultivar with or without Al treatment. However, the authors also noted that the possible precipitation of insoluble Al-phosphate could have caused a P deficiency that in turn may have triggered citric acid excretion. Pellet et al. (1995) observed in maize (*Zea mays L.*) that Al exposure stimulated release of citrate and phosphate from the root apex of Al-tolerant genotypes but not Al-sensitive ones. In their short-term experiment, P deficiency was unlikely to occur, because seedlings were first grown in full nutrient solution containing 200 μM P for 5 days, and then exposed to simple salt ($CaCl_2$) solution with Al^{3+} for up to 20h.

Hence, they concluded that Al-induced release of citrate could be an important aspect of differential Al tolerance in maize cultivars. In addition, they suggested that P exudation, which could have precipitated Al as Al-phosphate in the root cell wall, might be another important component of Al tolerance.

Research that does not support the hypothesis that Al-tolerance is related to organic acid excretion was found by Blamey et al. (1990). In their experiment, they found no difference in the amount or proportion of organically complexed Al in solution between tolerant (*L. pedunculatus* cv. Grasslands Maku) and sensitive (*L. corniculatus* cv. Maitland) species of lotus. However, an important reason for the inability to detect differences between cultivars may be due to the rapid breakdown of organic ligands by microorganisms, because the experiment was not conducted under aseptic conditions.

Research in Support of Internal Resistance Mechanisms Involving Organic Acids:

There is also evidence supporting the internal resistance mechanisms that involve the chelation and detoxification of Al by organic acids. Cambraia et al. (1983) studied the effects of Al on organic acid composition of the root system of sorghum and found that the tolerant cultivar accumulated significantly higher amounts of trans-aconitate (3.5 times greater) and malate (2 times higher) than the sensitive one under Al stress. Lee and Foy (1986) reported that an Al-tolerant snapbean cultivar contained higher concentrations of total organic acids than the Al-sensitive one, either in the presence or absence of Al stress, indicating that differences existed between cultivars prior to the imposition of stress. Further, Foy

et al. (1987) found that Al stress significantly reduced concentrations of citric, succinic, and total organic acids in roots of the Al-sensitive cultivar of barley, but not in those of the Al-tolerant cultivar. Their results showed that the sensitive cultivar contained higher concentrations of citric, malic, succinic, levulinic, and total organic acids than did the tolerant one when grown in the absence of Al. However, when exposed to Al, the tolerant cultivar maintained higher concentrations of organic acids in its roots than did the sensitive one. This ability to maintain higher concentrations of organic acids was concluded by Foy et al. (1987) to be correlated to the superior Al tolerance in the tolerant cultivar. These results suggested that Al was detoxified by an internal chelation mechanism, or that Al tolerance is correlated with the maintenance of higher concentrations of organic acids within the plants.

With some exceptions (Cambraia et al., 1983), the data of various researchers indicate that concentrations of organic acids decline under Al stress, with Al tolerant cultivars maintaining higher tissue concentrations of organic acids than sensitive ones (Foy et al., 1987; Lee and Foy, 1986; Suhayda and Haug, 1986). However, in contrast to this general trend, Foy et al. (1990) found that concentrations in roots of total organic acids in both tolerant and sensitive cultivars of wheat increased under Al stress, with Al tolerant cultivars containing lower concentrations of malic acid and total organic acids than sensitive ones with or without Al. This result is not surprising, however, considering recent evidence by Delhaize et al. (1993) that Al-tolerant wheat cultivars exude malic acid into the rhizosphere under Al stress.

While chelation of Al in the cytoplasm or rhizosphere may be an effective means of reducing the activity of phytotoxic Al species, there are concerns about the high energetic cost to the organism involved in the production and exudation of chelating agents (Taylor, 1987; 1988). For example, organic acids excreted by plants grown in soil can be readily and rapidly degraded by microorganisms, so plants need to continue to excrete these products. Some researchers questioned whether the magnitude of excretion is sufficient to protect plants. However, it is not necessary that Al in the bulk soil be chelated for a plant to be tolerant of Al. The levels of energetic cost may be physiologically relevant, because root exudates are localized primarily within the apoplasm, and it is necessary to protect only the sensitive root tip for a short period of time before maturation occurs (Ryan et al., 1993).

Taro: An Important Food Crop on Tropical Acid Soils

Taro [*Colocasia esculenta* (L.) Schott], a member of the *Araceae* family, is an ancient crop grown throughout the humid tropical and sub-tropical regions of the world for its edible corms and leaves (Wang, 1983). In the Pacific islands, the crop attained major importance in diets of their inhabitants (de la Pena, 1970). However, taro is an internationally underexploited crop (National Academy of Sciences, 1975) that has not been given much attention scientifically (Sunell and Arditti, 1983). The nutritional value of taro in the human diet has been studied by some researchers and is believed to be superior over other starchy staples in certain respects. The

size of the taro starch grain is one-tenth that of potato (Payne et al.,1941) and its digestibility has been estimated to be 98.8 percent (Langworthy and Deuel, 1922; Potgieter, 1940). Therefore, taro is an ideal food for people with digestive problems (Potgieter, 1940). High vitamin content of taro is believed to be related to good teeth development, among other healthful benefits (Wang, 1983). In an investigation of teeth among infants of oriental stock in Hawaii, those who received rice as their main carbohydrate showed a higher incidence of dental decay compared to those of the same ancestry fed a diet where taro replaced rice (Larsen et al., 1934; Potgieter, 1940).

The potential exists to breed for improved taro cultivars, because of the remarkable variability of various chemical constituents in taro seedlings, such as calcium oxalate in leaves, percent protein in corms, etc. (Strauss et al., 1982; Sunell and Arditti, 1983). However, before any breeding programs can be successfully implemented, there is a need to elucidate the functions of these variable constituents in taro plants, with emphasis on their roles in human nutrition and involvement in Al resistance.

Research on Response of Taro Cultivars to Aluminum Toxicity

Differential response to Al-toxicity has been found to exist within the taro germplasm (Miyasaka et al., 1993a, 1993b). The variability in response to Al toxicity is a useful characteristic in the study of Al resistance mechanism in taro plants. It is possible that tolerance to acid soils in taro cultivars can be improved through

breeding, tissue and cell culture, and selection (Sunell and Arditti, 1983). Oxalic acid has been found in substantial quantities in taro leaves and corms (Standal, 1983), and has been suggested to be related to Al-tolerance in taro (Calisay, 1996), as will be discussed later.

Oxalate in Taro:

1) Oxalate:

Oxalate is a common constituent that is produced in many crop plants and pasture weeds. It accumulates primarily as soluble oxalate, insoluble calcium oxalate, or a combination of these two forms, depending on species (Libert and Franceschi, 1987). In taro, oxalate content varies with cultivar (Sunell and Arditti, 1983). Oxalic acid is the simplest of the dicarboxylic acids. It is both a relatively strong acid and a reducing agent. Salts of oxalate are sparingly soluble due to the strong chelating capacity of the divalent oxalate ion, e.g., the solubility of calcium oxalate is only 6.0 mg L^{-1} at 18 ^{0}C (Libert and Franceschi, 1987).

The exact biosynthetic pathways of oxalate in the overall metabolism of the plant are not established, and it is likely that several pathways exist in higher plants (Libert and Franceschi, 1987). Although there has been demonstration that oxalate is formed from precursors synthesized in the light, and major precursors include glycolate and L-ascorbic acid (Franceschi and Horner, 1980), it is disputable that photorespiration is a prerequisite for oxalate synthesis (Libert and Franceschi, 1987). During the conversion of glycolate to oxalate, glycolate is first oxidized to form glyoxylate. The same enzyme (glycolate oxidase) in this step then catalyzes

the oxidation of glyoxylate to oxalate in the second step (Libert and Franceschi, 1987). The enzymatic basis of the conversion from L-ascorbic acid to oxalate has not yet been determined. Oxalate synthesis could occur in peroxisomes or the cytoplasm, and its transport in the phloem is possible (Libert and Franceschi, 1987).

Since examples of oxalate breakdown *in vivo* are scarce, and the process of conversion of oxalate to other carbon compounds is slow, oxalate is generally considered to be an end product of metabolism (Franceschi and Horner, 1980). However, a plant enzyme capable of oxalate oxidation has been detected in various tissues of *Begonia semperflorens (*Sasaki, 1963). Oxalic acid oxidase was associated with chloroplasts and was postulated to oxidize oxalate to hydrogen peroxide and CO_2. In addition, the decarboxylation of oxalate was suggested by Franceschi (1987), who demonstrated the incorporation of ^{14}C labelled oxalic acid into starch in *Lemna minor L.* plants, and speculated that starch was synthesized through re-fixation of CO_2 released from the decarboxylated oxalate.

2) Calcium oxalate:

Calcium oxalate crystals are widely distributed in various tissues and organs in plants of a majority of plant families (Esau, 1965; Franceschi and Horner, 1980), among them the family *Araceae*. These crystals are of two chemical compositions: calcium oxalate monohydrate ($CaC_2O_4.H_2O$), which is monoclinic in form, and calcium oxalate polyhydrate ($CaC_2O_4.nH_2O$), which is tetragonal in form (Tang and Sakai, 1983). The solubility and instability of calcium oxalate increase with the level of hydration: the trihydrate being most soluble and unstable, followed in order by the

dihydrate and the monohydrate (Libert and Franceschi, 1987). Calcium oxalate crystals may occur as prisms, druses, crystal sand, raphides, and styloids. They can be found in cells resembling those neighbouring them but lacking crystals, or they may be confined to special crystal-containing cells called idioblasts. Crystals are formed within vacuoles and are surrounded by an envelope (Fahn, 1982).

Calcium oxalate crystals in taro exist in two forms: druses (80-95% of the total) and raphides (Sunell and Arditti, 1983). Little is known of the factors affecting raphide and druse formation, or why one form develops instead of the other (Tang and Sakai, 1983). The density of crystals in corms may be as high as 120,000 cm^{-3}, and even higher in leaves (Sunell and Healey, 1978; Gueguen, 1908; as reported by Sunell and Arditti, 1983).

3) Functions of Oxalate:

Several possible functions of oxalate have been proposed. First, the occurrence of calcium oxalate raphides has been thought to provide protection for plants against foraging animals. In taro, for example, the irritation and piercing sensation of the mouth and throat experienced during ingestion is thought to be caused by the physical characteristics of the needle-like crystals (Sakai and Hanson, 1974). These raphides exhibit three structural features which are thought to be linked to the irritation: First, the crystals have two distinct points, one tapering to an elongate point, and the other abruptly pointed. Second, the crystals have surface barbs oriented away from the tapering point. And third, deep grooves are present along the length of the crystals. The tapering points, barbs, and grooves

thus form a mechanical mechanism in penetrating the epithelial cells of the mouth and throat (Sakai and Hanson, 1974). However, many nonacrid plants also contain needle-like raphides with pointed ends, and some acrid plants, such as palms (*Arenga* and *Ptychosperma*) contain raphides without barbs or grooves (Sakai, 1979). In addition, since cooking has no effect on the structure of barbs and grooves of raphides in *Colocasia* but removes most of its acridity (Tang and Sakai, 1983), some doubt has been cast on attributing raphide crystals as the sole cause of acridity. Consequently, suggestions of the toxic effect of oxalate itself and other acrid chemicals to livestock have been made (Franceschi and Horner, 1980; Tang and Sakai, 1983).

The second proposed function of oxalate is that oxalic acid is produced to regulate the raised pH of the cell sap during the uptake and reduction of nitrate (Franceschi and Horner, 1980). The precipitation of oxalic acid as insoluble calcium oxalate may serve to restore ionic balance and reduce osmotic pressure following the neutralization of OH^- (Franceschi and Horner, 1980). In addition, soluble salts of oxalate may play an important role in osmoregulation (Libert and Franceschi, 1987).

The third function attributed to oxalate is that of the regulation of calcium levels in plants. The following model is proposed by Franceschi (1989): when excess apoplastic Ca reaches some critical level so that passive influx exceeds active efflux and compartmentation, a signal involving calmodulin is triggered to induce the formation of insoluble Ca oxalate, thus removing the excess Ca from the

apoplast. On the other hand, under conditions where Ca is limited, Ca oxalate crystals are dissolved to provide Ca for growth and cell maintenance (Franceschi, 1989). Similarly, Borchert (1990) found in *Carya ovata* leaves that rising apoplastic Ca can act as a developmental signal inducing transdifferentiation of mesophyll cells into crystal cells, which serve as Ca^{2+} sinks by precipitating absorbed Ca^{2+} as Ca oxalate (Borchert, 1990).

4) Oxalate: A Role in Aluminum Resistance in Taro?

In a recent study on differential response of taro cultivars to Al toxicity, Calisay (1996) reported that increasing Al levels in solution significantly decreased concentrations of total oxalate in roots of four cultivars. However, the Al-tolerant 'Lehua maoli' maintained the highest level of total oxalates in roots at 1330 μM Al in solution compared to 'Bun-long' and two other less tolerant cultivars. In a second experiment, increasing Al levels in solution significantly decreased concentrations of both water soluble and insoluble oxalates in roots of both taro cultivars (Calisay, 1996). At 890 μM Al and 1000 μM Ca, the Al-tolerant 'Lehua maoli' contained a significantly greater concentration of insoluble oxalates in roots than the Al-sensitive 'Bun-long'. No significant cultivar differences in water soluble oxalate concentrations in roots were found for the two cultivars.

In comparing the Al-tolerant 'Lehua maoli' and Al-sensitive 'Bun-long', Calisay (1996) found that Al concentrations in roots, petioles, and leaf blades significantly increased in response to elevated Al levels, however, Al-tolerant 'Lehua maoli' had a significantly higher Al concentration in roots and a significantly lower Al

concentration in leaf blades at 890 μM Al and 1000 μM Ca, compared to Al-sensitive 'Bun-long'. Further, Calisay (1996) examined the possible formation of Al-oxalate in the roots of tolerant taro 'Lehua maoli'. Scanning electron microscope energy dispersive X-ray florescence analysis indicated the formation of a solid, non-crystalline, organic compound containing Al. In addition, oxalate decarboxylase enzyme analysis indicated that under the catalytic action of decarboxylase, an enzyme specific for the breakdown of oxalate to CO_2, Al was cleaved from oxalate complexes in roots of 'Lehua maoli'.

With the use of ^{27}Al NMR (Nuclear Magnetic Resonance), three forms of Al-oxalate complex under aqueous condition have been reported (Sjoberg and Ohman, 1985; Thomas et al., 1991; Kerven et al., 1995): 1) 1:1 bidentate $Al(C_2O_4)^+$; 2) 1:2 bidentate $Al(C_2O_4)_2^-$; and 3) 1:3 bidentate $Al(C_2O_4)_3^{-3}$.

Based upon these findings, Calisay (1996) suggested that: 1) high tolerance in taro might be related to the capacity of the plant to maintain higher levels of oxalate under Al stress; 2) it is possible that oxalate in roots forms complexes with Al, thereby detoxifying Al and preventing its further movement into leaf blades; and 3) there exists the possibility of exudation of oxalate from taro roots into the rhizosphere, because the concentration of water soluble oxalates decreased with increasing Al levels.

Chapter 3

Organic Acid Exudation of Two Taro Cultivars Under Aluminum Stress

Abstract

Aluminum(Al) toxicity is a major factor limiting plant growth in acid soils. One proposed mechanism of Al tolerance in plants is the release of Al-chelating compounds into the rhizosphere. To examine the role of exudation of oxalate and/or other organic acids in Al tolerance, two taro [*Colocasia esculenta* (L.) Schott] cultivars ('Lehua maoli' and 'Bun-long') were grown under aseptic conditions in the presence and absence of Al in nutrient solution. Both cultivars excreted significantly more oxalate into the culture solutions under 900 μM Al than 0-Al treatment. The amount of oxalate excreted increased as Al concentration in solution was increased. No detectable amounts of citrate, malate, or succinate were found in root exudate. Addition of 900 μM oxalate to nutrient solutions containing 900 μM Al restored root growth of 'Lehua maoli' and 'Bun long' to 96% and 66%, respectively, compared to controls (0-Al, no oxalate). The exudation of oxalate was specifically a response to Al stress rather than phosphorus deficiency. Oxalate is known to be a strong chelator of Al. Oxalate exudation from taro roots under Al stress appears to be an important aspect of Al tolerance in taro.

Materials and Methods

Tissue-cultured plantlets of two taro cultivars, i.e., 'Bun-long' and 'Lehua maoli', were obtained from a commercial laboratory following the method of Keolanui et al. (1993). Plantlets were ready for experiments when they were about 10 cm tall.

Plantlets were grown under aseptic conditions to quantitatively estimate their production of organic acids. Under non-aseptic conditions, it is possible that exudates can be rapidly metabolized by micro-organisms.

At the conclusion of each experiment, a subsample of 0.1 mL of the medium from each growth vessel was plated onto nutrient agar, incubated for 3 days, and examined for microbial contamination to check whether aseptic conditions were maintained throughout the experiment. Any contaminated replicates were noted and results compared with non-contaminated samples. Fresh weights of shoots and roots were also determined.

The basal nutrient solution was a modified Steinberg solution (Miyasaka et al., 1993a). The macronutrient concentrations were, in mM: NH_4-N, 1.2; NO_3-N, 3.6; P, 0.1; K, 1.2; Ca, 1.0; Mg, 0.4; S, 0.7; The micronutrients were, in μM: Mn, 2; B, 6; Zn, 1; Cu, 0.5; Mo, 0.1; Fe (as FeEDDHA), 10. All supplies and the basal nutrient solution were autoclaved, except for $AlCl_3$ solution which was filter-sterilized through 0.2 μm pore diameter syringe filters to avoid Al precipitation due to pH changes during autoclaving. To make one liter of Al stock solution (0.09 M), $AlCl_3$ was added to 400 mL of deionized water pre-acidified to pH 4.0 with 0.1N

HCl. Solution pH was then raised slowly with 0.15 M NaOH to approximately pH 3.87 and brought to 1 liter with deionized water.

The pH of basal nutrient solution was adjusted initially to 4.0 with 0.1N HCl before autoclaving. The addition of filter-sterilized $AlCl_3$ solution to autoclaved basal nutrient solution lowered its pH to around 3.6 for an initial Al level of 900 µM. An appropriate amount of 0.1 N autoclaved NaOH was added to bring solution pH back to around 4.0. Since pH levels may play a role in the effect of Al on root growth, final solution pH was also recorded to determine if pH was maintained at 4.0 throughout the experiment.

Prior to treatment, plantlets were rinsed three times with 15 mL of sterile deionized water each time to remove substances that may have accumulated on root surfaces over the previous growth period. In all four experiments, plantlets of similar weights were grouped into the same replicate. All experiments followed a randomized complete block design to remove variability due to plant sizes, light condition, and temperature. Growth vessels were kept in a growth chamber with temperature between 22-28 ^{0}C, 65% relative humidity, and 24h light. The light intensity was 45 µmol·m^{-2}·s^{-1}.

Time Course Experiment

To determine whether oxalic acid is released under Al stress, and how this release varies with time, a time course experiment was implemented. Tissue-cultured plantlets of two taro cultivars, i.e., 'Bun-long' and 'Lehua maoli', were grown in sterile culture for 10 days in the absence or presence of 900 µM Al. For

each treatment, two plantlets were grown in 50 mL of treatment solution. There were four replicates of a factorial combination of two Al levels and two taro cultivars.

Samples of growth medium were taken on days 3, 5, 7, and 10 after treatments were imposed. At each sampling time, 1 mL of solution was withdrawn from each growth vessel using a sterile pipette, diluted with 1 mL of sterile deionized water, and then stored in a freezer until analyses for oxalic acid.

Dose Response Experiment

In the dose response experiment, the effect of Al on exudation of oxalate and other organic acids was studied. Two taro cultivars were exposed to four initial Al levels (in μM: 0, 300, 600, 900). For each treatment, three plantlets were grown in a growth vessel containing 100 mL treatment solution. Treatment design was a complete factorial design of two taro cultivars x four Al levels, with five replicates. Solution samples were taken for the analyses of oxalate and other organic acids (succinic, malic, and citric) one week after exposure to Al treatments.

Experiment on Ameliorative Effect of Oxalate

To test the ameliorative effect of oxalate on root growth inhibition caused by Al toxicity, roots of 'Bun-long' and 'Lehua maoli' were removed and plantlets were grown in sterile nutrient solution for two days to allow healing of wounds. Plantlets were then transferred to treatment solutions, with five replicates per treatment. Each plantlet was grown in 15 mL solution in a test tube under aseptic conditions. Treatment design was:

1. control: basal nutrient solution;

2. basal nutrient solution + 900 μM Al;

3. basal nutrient solution + 900 μM Al + 300 μM oxalic acid;

4. basal nutrient solution + 900 μM Al + 600 μM oxalic acid;

5. basal nutrient solution + 900 μM Al + 900 μM oxalic acid;

6. basal nutrient solution + 900 μM Al + 1200 μM oxalic acid;

7. basal nutrient solution + 900 μM Al + 1500 μM oxalic acid;

At the end of 14 days, when visible differences in root growth due to treatments were observed, plantlets were harvested, and root elongation was measured.

Phosphorus Experiment

To investigate whether the exudation of oxalate was also related to low phosphorus (P) in addition to Al stress, tissue-cultured plantlets of 'Bun-long' and 'Lehua maoli' were grown under sterile conditions in the presence or absence of 900 μM Al containing three levels of P (in μM: 0, 0.3, and 100). Treatment design was a complete factorial of two taro cultivars x two Al levels x three P levels. Each treatment was replicated 5 times, with one plantlet grown in a culture tube containing 15 mL nutrient solution per replicate. One week after exposure to treatments, solution samples were taken and analyzed for oxalate.

Ion Chromatography

Samples of the medium were analyzed for oxalic acid and other organic acids using an HPLC (Dionex DX 500 Chromatography System, Dionex

Corporation, Sunnyvale, CA) with an anion self-regenerating suppressor (ASRS-I 4mm, Dionex Corp.). For oxalate analysis, an ion-exchange analytical column (IonPac AS4A-SC 4mm, Dionex Corp.) and a guard column (IonPac AG4A-SC 4mm, Dionex Corp.) were used with an eluent of 22mM sodium borate / boric acid at a flow rate of 2.0 mL min^{-1}. For analyses of other organic acids, e.g., succinic, malic, and citric, an anion exchange column (IonPac AS10-4mm, Dionex Corp.) and a guard column (IonPac AG10-4mm, Dionex Corp.) were used with 50 mM NaOH as eluent for analyses of succinic and malic, and 100 mM NaOH as eluent for analysis of citric. The flow rate was 1.0 mL min^{-1}. Concentrations of organic acids were determined via measurement of electrical conductivity by a CD20 Conductivity Detector (Dionex Corp., Sunnyvale, CA).

Statistical Analyses

Statistical analyses of the data were conducted using the SAS computer program (Statistical Analysis Systems Institute, 1982). Analysis of variance (ANOVA) was calculated to test treatment effects. In general linear models (GLM) procedure, regression models were determined for the time course and dose response experiments. Single degree of freedom contrasts were used to evaluate the linear, quadratic, and cubic effect of Al on the exudation of oxalate. The relationship between oxalate exudation and solution Al levels was further examined by linear and nonlinear regression analyses. A probability level of 0.05 or less was considered to be statistically significant.

Results and Discussion

Experiment 1: Time course experiment.

There existed a significant cultivar difference in initial fresh weight of taro plantlets (Table 3.1). At the end of the experiment, there were also significant cultivar differences in shoot and root fresh weights; however, there was no significant Al effect (Table 3.1).

Table 3.1. Initial and final fresh weight of taro plantlets.

			Final fresh weight (g)	
Cultivar	Al (μM)	Initial fresh weight (g)	shoot	root
Bun-long	0	6.60 (0.58)*	3.67 (0.41)	1.78 (0.24)
Bun-long	900	6.98 (0.92)	3.61 (0.55)	1.94 (0.18)
Lehua maoli	0	4.60 (0.79)	2.33 (0.44)	1.30 (0.29)
Lehua maoli	900	4.48 (0.91)	2.27 (0.59)	1.21 (0.23)
Analysis of Variance				
Source			Pr>F	
Al		0.5100	0.6300	0.6600
Cultivar		0.0001	0.0001	0.0001
AlxCultivar		0.2200	0.9900	0.1200
Rep		0.0001	0.0001	0.0001

*. Means are followed by standard errors of mean in parentheses.

Aluminum in solution stimulated the excretion of oxalic acid from roots of both taro cultivars (Table 3.2; Fig. 3.1). By 3 d after start of treatments, both cultivars exuded significantly more oxalic acid under 900 μM Al than 0-Al treatment (P=0.0039). No cultivar difference in oxalic acid exudation was found (P=0.14). The cumulative amount of oxalic acid concentrations excreted by both cultivars in

response to 900 μM Al increased linearly up to 7 days after initiation of treatments (Table 3.3; Fig. 3.1). In 0-Al treatment, however, both cultivars released only small amounts of oxalic acid, and the exudation changed only slightly over time (Fig. 3.1).

Table 3.2. Oxalate exudation from two taro cultivars under two Al levels over ten days.

		Days after start of treatment			
Cultivar	Al(μM)	3	5	7	10
		Oxalate in solution (μM)			
Bun-long	0	6.18 (6.18)*	13.82 (5.42)	11.55 (6.74)	10.27 (3.89)
Bun-long	900	47.10 (3.83)	74.92 (5.53)	86.80 (10.86)	86.45 (18.83)
Lehua maoli	0	0.00 (0.00)	11.18 (3.73)	10.16 (3.57)	5.65 (3.31)
Lehua maoli	900	13.77 (7.98)	31.76 (2.86)	34.30 (4.23)	33.21 (3.90)

*. Means are followed by standard errors of mean in parentheses.

Table 3.3. Change of oxalate exudation with time over 7 days (y: in μg/g root fresh weight, refers to oxalate exuded from taro roots. X: in days, refers to the number of days after start of treatments).

Cultivar	Regression model	r^2
Bun-long	y=28.29X+12.35	0.92
Lehua maoli	y=20.49X+3.38	0.56

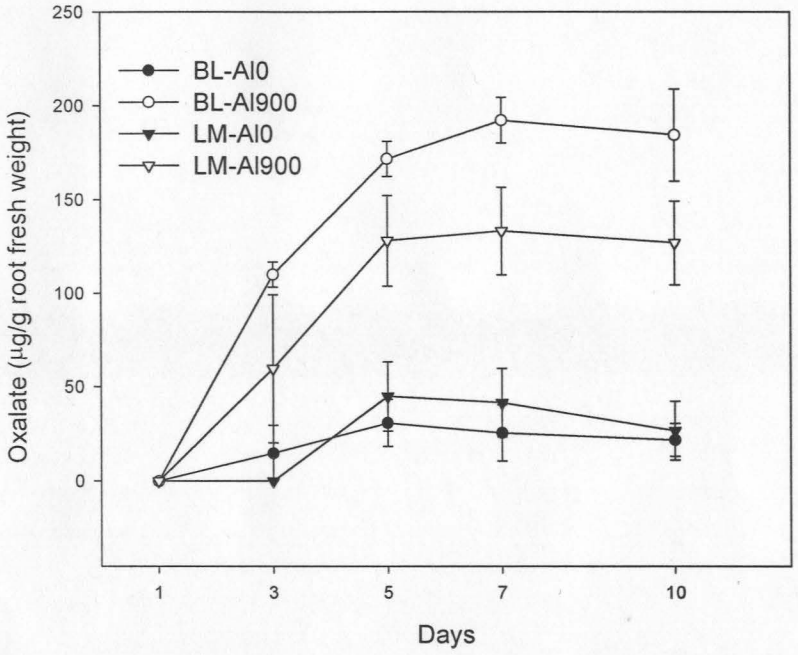


Fig 3.1. Exudation of oxalate from two taro cultivars [Bun-long (BL) and Lehua maoli (LM)] at two levels of Al [0 (Al0) and 900 (Al900) µM] over a period of 10 days.

Studies on the time course of organic acid exudation from other crop species under Al stress have shown a similar linear trend over time. In wheat, Al-stimulated excretion of malic acid can be detected as early as 15 min after exposure to Al, and the cumulative amount of malic acid excreted by wheat seedlings in response to 50 μM Al was linear over 24 h for both Al-tolerant and Al-sensitive genotypes, with the former excreting 5- to 10-fold more malic acid than the latter (Delhaize et al., 1993). In another study with wheat, cultivar differences in the effect of Al on malate accumulation were detected between 24 and 72 h after exposure to Al, and accumulation of malate was essentially linear with time over 5 d (Basu et al., 1994a). In maize, the rate of citrate exudation increased significantly in the presence of 6 μM Al compared to 0-Al treatment from 24 to 48 h in an Al-tolerant cultivar (Pellet et al., 1995). These studies show that enhanced exudation of organic acids from different crop species under Al stress may be expressed from within a period of minutes or hours to over a week. The difference in the length of time of continued exudation of organic acids may reflect differences in plant species, plant age, volume of nutrient solutions and Al levels used in the studies.

Since no further increases in release of oxalate in nutrient solutions were observed after 7 d of growth for both taro cultivars under Al stress, plants were allowed to grow in nutrient solutions in a subsequent dose response experiment for seven days before collecting solution samples. Thus, a maximum concentration of oxalate accumulation was obtained for detection.

Sterility check of growth solutions indicated that no development of microbial colonies was evident for most solution samples. For a few samples in which contamination was found, the amount of oxalic acid detected in root exudate was not greatly affected compared to non-contaminated samples. No significant difference in final solution pH was found for the two cultivars or Al levels (Table 3.4).

Table 3.4. Final solution pH.

Cultivar	Al (μM)	pH
Bun-long	0	3.72 (0.14)[1]
Bun-long	900	3.93 (0.32)
Lehua maoli	0	3.71 (0.21)
Lehua maoli	900	4.02 (0.16)

Analysis of Variance

Source	Pr>F
Al	0.4700
Cultivar	0.5600
AlxCultivar	0.4800
Rep	0.4600

1. Means are followed by standard errors of mean in parentheses.

Experiment 2: Dose response experiment.

The two taro cultivars differed significantly in initial fresh weight (Table 3.5). Shoot and root fresh weights were also significantly different at the end of the experiment (Table 3.5). No significant Al effect was found in final fresh weight (Table 3.5).

Table 3.5. Initial and final fresh weight of taro plantlets.

Cultivar	Al (μM)	Initial fresh weight (g)	Final fresh weight (g) shoot	root
Bun-long	0	11.09 (0.83)*	4.66 (0.26)	3.43 (0.26)
Bun-long	300	12.45 (0.93)	5.69 (0.46)	3.71 (0.13)
bun-long	600	12.14 (0.25)	5.37 (0.19)	3.19 (0.13)
Bun-long	900	12.02 (0.42)	4.40 (0.39)	3.50 (0.26)
Lehua maoli	0	5.26 (0.36)	2.44 (0.05)	1.95 (0.17)
Lehua maoli	300	8.54 (0.46)	4.22 (0.32)	2.27 (0.13)
Lehau maoli	600	8.01 (0.83)	3.55 (0.10)	2.14 (0.21)
Lehua maoli	900	7.24 (0.75)	3.02 (0.31)	1.79 (0.21)

Analysis of Variance

Source	Pr>F		
Al	0.0620	0.0640	0.2200
Cultivar	0.0001	0.0001	0.0001
AlxCultivar	0.4400	0.4200	0.3600
Rep	0.2400	0.1400	0.1500

*. Means are followed by standard errors of mean in parentheses.

Increasing Al concentrations from 0 to 900 μM significantly increased exudation of oxalate into the rhizosphere for both cultivars (P=0.0001; Table 3.6; Fig. 3.2). However, there was no significant cultivar difference in oxalate exudation in response to increasing Al levels (P=0.65; Fig. 3.2). Accumulation of oxalate in the growth media was linear for 'Bun-long' over the range of solution Al levels tested (Table 3.7; Fig. 3.2); whereas for 'Lehua maoli', the increase of oxalate in root exudate with Al followed a cubic trend (Table 3.7; Fig. 3.2).

The increased exudation of oxalate in response to Al helps to explain the decrease of water soluble oxalate concentrations in taro roots with increasing Al levels, as reported by Calisay (1996). In this experiment, at 900 μM Al, 'Bun-long' and 'Lehua maoli' exuded oxalate at 5.13 $g \cdot kg^{-1}$ and 3.10 $g \cdot kg^{-1}$ root dry weight basis, respectively. Whereas at 890 μM Al, water soluble oxalate in roots decreased 2 to 2.2 g per kg root dry weight for 'Bun-long' and 'Lehua maoli' (Calisay, 1996). The minor differences in magnitude between oxalate exuded from taro roots as compared to the decrease of water soluble oxalate in taro roots might be due to the differences in plant sizes and Al levels used, or due to *de novo* synthesis of oxalate. No detectable amounts of citrate, malate, or succinate was found in nutrient solution.

Aluminum-stimulated exudation of organic acids was also demonstrated in wheat (Delhaize et al., 1993; Pellet et al., 1996). Malic acid excretion over 7h from 4-d-old seedlings of Al-resistant wheat genotypes 'Atlas' and 'ET3' increased

linearly in response to increasing levels of Al from as little as 5 μM to 20 μM, in contrast, Al-sensitive wheat genotypes 'Scout' and 'ES3' exhibited a low rate of malate exudation that was insensitive to Al exposure (Pellet et al., 1996).

Taro cultivar 'Lehua maoli' was found to be more tolerant of Al than 'Bun-long', when grown from 'hulis', which are vegetative propagating materials composed of approximately 20 cm of petioles and 0.5 cm of upper corm (Miyasaka et al., 1992; Calisay, 1996). However, tissue-cultured 'Bun-long' and 'Lehua maoli' did not exhibit significant differences in tolerance to Al toxicity (see chapter 4). This finding may be the reason that the two taro cultivars used in this experiment did not show a significant difference in exudation of oxalate in response to Al.

Table 3.6. Oxalate exudation from two taro cultivars grown under four Al levels over a seven day period.

	Initial solution Al (μM)			
Cultivar	0	300	600	900
	Oxalate in solution (μM)			
Bun-long	7.40 (2.61)*	36.96 (6.03)	51.19 (2.72)	72.29 (15.63)
Lehua maoli	1.06 (0.49)	35.92 (4.35)	30.58 (2.69)	28.29 (2.26)

*. Means are followed by standard errors of mean in parentheses.

Table 3.7. Linear, quadratic, and cubic effect of Al on oxalate exudation (y: in µg/g root fresh weight, refers to oxalate exuded from taro roots. X: in µM, refers to initial Al concentrations in solutions).

	Pr>F		
Cultivar	Linear (Al)	Quadratic (Al^2)	Cubic(Al^3)
Bun-long	0.0002	0.7200	0.8310
Lehua maoli	0.0001	0.0002	0.0043

Cultivar	Regression model	r^2
Bun-long	$y=0.19X+20.65$	0.58
Lehua maoli	$y=1.09x10^{-6}X^3-1.8x10^{-3}X^2+0.88X+4.26$	0.85

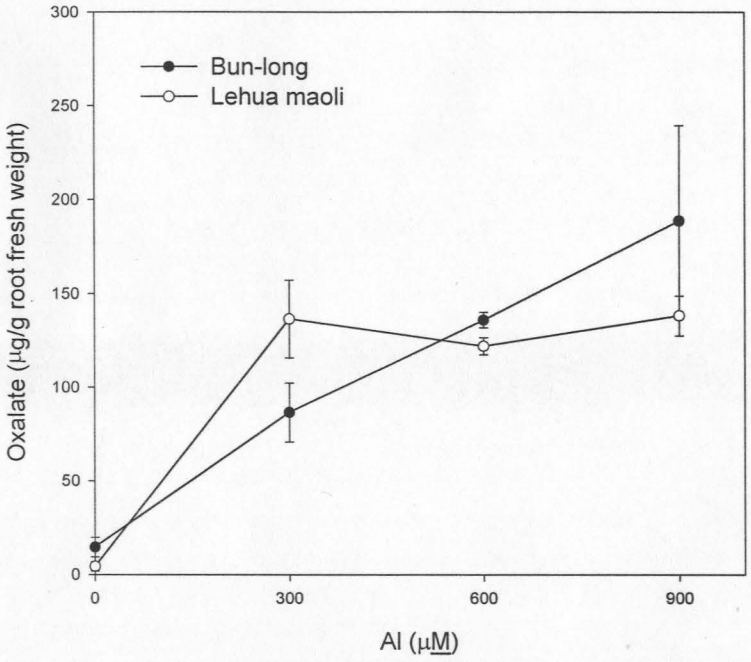


Fig 3.2. Effect of increasing initial Al levels in solution on the exudation of oxalate from two taro cultivars over seven days.

As in the previous experiment, a sterility check did not reveal any significant contamination of growth media during the experiment. Final solution pH ranged from 3.6 - 4.2. A significant cultivar difference and a cultivar x Al interaction in final solution pH was found (Table 3.8). Aluminum treatments generally caused a decline in pH, which was probably because of depressed plant growth in the presence of Al. A higher final solution pH for 'Bun-long' than for 'Lehua maoli' could be the result of a relatively greater growth of 'Bun-long' due to its greater initial weight (Table 3.5). Since increasing Al stimulated the excretion of oxalate, which is hypothesized to chelate with Al and therefore ameliorate its inhibition of plant growth, an increase in solution pH at a certain point might be expected as plant growth is improved due to increased exudation of oxalate (Table 3.8; Fig. 3.2). The cultivar x Al interaction for final solution pH might be related to differential growth rate due to differences in initial plant sizes and Al levels.

Table 3.8. Final solution pH.

	Al (μM)			
Cultivar	0	300	600	900
	pH			
Bun-long	4.0 (0.02)*	3.9 (0.12)	3.8 (0.15)	4.2 (0.09)
Lehua-maoli	3.8 (0.06)	3.6 (0.11)	3.7 (0)	3.6 (0.09)

Analysis of Variance

Source	Pr>F
Al	0.0840
Cultivar	0.0001
AlxCultivar	0.0021
Rep	0.0920

*. Means are followed by standard errors of mean in parentheses.

Experiment 3. Root growth inhibition by Al and amelioration of Al toxicity by oxalate.

Initial fresh weight of whole plants and final fresh weights of shoots and roots were significantly different for the two taro cultivars (Table 3.9).

Table 3.9. Initial and final fresh weights of taro plantlets.

Cultivar	Oxalate (μM)	Al (μM)	Initial fresh weight (g)	Final fresh weight (g) Shoots	roots
Bun-long	0	0	0.41 (0.08)*	0.36 (0.08)	0.09 (0.03)
Bun-long	0	900	0.49 (0.11)	0.28 (0.09)	0.01 (0.00)
Bun-long	300	900	0.36 (0.08)	0.20 (0.05)	0.01 (0.01)
Bun-long	600	900	0.35 (0.06)	0.22 (0.04)	0.02 (0.01)
Bun-long	900	900	0.38 (0.06)	0.33 (0.06)	0.07 (0.03)
Bun-long	1200	900	0.42 (0.05)	0.25 (0.04)	0.04 (0.02)
Bun-long	1500	900	0.42 (0.05)	0.25 (0.05)	0.05 (0.03)
Lehua maoli	0	0	0.78 (0.12)	0.69 (0.11)	0.11 (0.03)
Lehua maoli	0	900	0.90 (0.17)	0.65 (0.13)	0.02 (0.01)
Lehua maoli	300	900	1.06 (0.18)	0.77 (0.18)	0.05 (0.01)
Lehua maoli	600	900	0.96 (0.17)	0.80 (0.17)	0.11 (0.02)
Lehua maoli	900	900	1.20 (0.19)	0.77 (0.13)	0.15 (0.03)
Lehua maoli	1200	900	1.00 (0.23)	0.74 (0.16)	0.07 (0.02)
Lehua maoli	1500	900	0.99 (0.09)	0.66 (0.10)	0.07 (0.02)

Analysis of Variance

Source		Pr>F	
Cultivar	0.0001	0.0001	0.0006
Oxalate	0.7800	0.9600	0.0003
Cultivar x Oxalate	0.5000	0.7900	0.3000
Rep	0.0009	0.0006	0.2200

*. Means are followed by standard errors of mean in parentheses.

Root elongation was severely inhibited in the presence of 900 μM Al in solution during the two-week growth period for the two taro cultivars, with 91% and

73% inhibition for 'Bun-long' and 'Lehua maoli', respectively (Table 3.10). However, no significant cultivar difference was found in root inhibition induced by Al (Table 3.10).

Table 3.10. Inhibition of root elongation under Al stress.

Cultivar	Al(μM)	Root-growth inhibition(%)*
Bun-long	0	0
Bun-long	900	91
Lehua maoli	0	0
Lehua maoli	900	73

Analysis of Variance

Source	Pr>F
Cultivar	0.1000
Al	0.0001
Cultivar x Al	0.1000
Rep	0.4400

*. Root-growth inhibition(%)=[1-(root length in Al/root length without Al)]x100

Addition of oxalate to nutrient solutions containing 900 μM Al significantly ameliorated inhibition of root elongation under Al stress for both cultivars (P=0.0001; Fig. 3.3). Addition of 600 μM and 900 μM oxalate restored root growth to 96% and 92%, respectively, of the growth level of control (0-Al, no oxalate) for 'Lehua maoli'; whereas for 'Bun-long', 900 μM oxalate restored root growth to 66% of control level. Oxalate concentrations above 900 μM began to reverse its ameliorative effect (Fig. 3.3), probably due to its detrimental effect on growth at high levels. This result suggested that a 1:1 ratio of oxalate to Al was the most effective in ameliorating Al toxicity, and that the fraction of oxalate in solution left

unbound with Al probably suppressed root growth of taro plantlets. The detrimental effect of oxalate on root growth was reported in sorghum (Shuman et al., 1991). Oxalate concentration of 10 μM decreased root length of sorghum seedlings to 80% of the level of control in the absence of Al, although in the presence of Al, oxalate was also effective in restoring root growth by chelating with Al and thereby detoxifying it (Shuman et al., 1991).

Root elongation was significantly greater for 'Lehua maoli' than for 'Bun-long' ($P=0.0008$) under 900 μM Al and the same oxalate levels; in other words, 'Lehua maoli' did not appear to need as much external oxalate to protect it against Al toxicity as 'Bun-long' (Fig. 3.3). Therefore, part of the Al may be complexed with oxalate and detoxified in the roots. This phenomenon might be the reason that 'Lehua maoli' exhibited a lower exudation of oxalate as found in previous time course and dose response experiments.

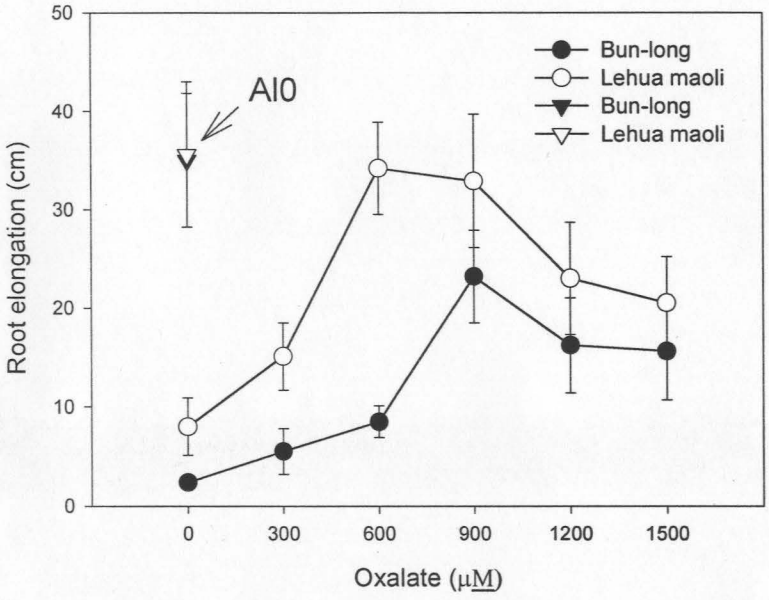


Fig 3.3. Ameliorative effect of oxalate on inhibition of root elongation under 900 μM Al.

The ameliorative effect of organic acids on the inhibition of root growth caused by Al toxicity is well documented (Hue et al., 1986; Ownby and Popham, 1989; Shuman et al., 1991; Delhaize et al., 1993; Basu et al., 1994a; Ryan et al., 1995; Pellet et al., 1996). Addition of exogenous citrate or malate to nutrient solution containing toxic levels of Al has been shown to effectively protect root growth and reduce Al toxicity in Al-sensitive wheat cultivars (Ownby et al., 1989; Delhaize et al., 1993; Basu et al.,1994a; Ryan et al., 1995; Pellet et al., 1996). According to Hue et al. (1986), malate is a moderate detoxifier and both citrate and oxalate are strong detoxifiers of Al. Therefore, it is not surprising that malate, citrate or oxalate exuded by plants under Al stress play an important role in ameliorating Al toxicity.

The magnitude of concentration of oxalate added to nutrient solutions to restore root growth to a control level was about 12 to 20-fold greater than found exuded by 'Bun-long' and 'Lehua maoli', respectively, in the previous time course and dose response experiment (Tables 3.2 and 3.6). This result raised the question of whether the magnitude of oxalate exudation detected earlier was relevant to the protection of roots under Al stress. First, in this experiment, the roots of taro plantlets were completely removed at the beginning, in order to facilitate measurement of root elongation under aseptic conditions. Final root elongation can be measured with ease only once at the end of the experiment, thus avoiding the possible introduction of contamination to the culture solution.

Under such circumstances, however, it is possible that plants would require a higher concentration of Al-chelator for an initial root growth, compared to plants with intact roots. This possibility was also reflected in the prolonged growth period before visible differences in root elongation due to Al treatment was observed. Second, in maize and wheat, it has been demonstrated that Al-induced organic acid release is localized to the root apex, where the primary symptoms of Al toxicity also occur (Ryan et al., 1993; Delhaize et al., 1993; Pellet et al., 1995). Thus, only a small portion of the root needs to be protected. A higher oxalate concentration may be expected to occur in the unstirred layer of solution surrounding the root apex, which should provide sufficient protection of the sensitive cells at root tip via chelation of Al in the rhizosphere.

Strict sterile conditions were maintained during the period of the experiment, and no contamination of the growth media was detected by sterility check at the conclusion of the experiment.

Experiment 4: Role of low phosphorus in oxalate exudation in taro.

The initial weights of the two taro cultivars were significantly different (Table 3.11). Cultivar differences in final shoot and root fresh weights were also significant (Table 3.11). However, no significant phosphorus or Al effects were found in final shoot and root fresh weights for the two cultivars (Table 3.11).

Table 3.11. Initial and final fresh weight of taro plantlets.

Cultivar	Al (μM)	P (μM)	Initial fresh weight (g)	Final fresh weight (g) shoot	root
Bun-long	0	0	1.51 (0.22)*	0.68 (0.16	0.27 (0.04)
Bun-long	0	0.3	1.54 (0.18)	0.62 (0.06)	0.21 (0.04)
Bun-long	0	100	1.58 (0.20)	0.70 (0.10)	0.23 (0.04)
Bun-long	900	0	1.61 (0.19)	0.62 (0.11)	0.22 (0.06)
Bun-long	900	0.3	1.62 (0.20)	0.69 (0.11)	0.22 (0.05)
Bun-long	900	100	1.67 (0.20)	0.85 (0.08)	0.20 (0.04)
Lehua maoli	0	0	2.13 (0.64)	0.88 (0.20)	0.51 (0.24)
Lehua maoli	0	0.3	2.19 (0.59)	0.65 (0.11)	0.63 (0.27)
Lehua maoli	0	100	2.10 (0.54)	0.73 (0.15)	0.61 (0.26)
Lehua maoli	900	0	2.16 (0.61)	0.88 (0.21)	0.44 (0.16)
Lehua maoli	900	0.3	2.22 (0.60)	0.89 (0.19)	0.49 (0.21)
Lehua maoli	900	100	2.24 (0.65)	0.87 (0.20)	0.44 (0.21)

Analysis of Variance

Source	Pr>F		
Phosphorus (P)	0.9500	0.3900	0.9400
Al	0.5700	0.0580	0.2500
Cultivar	0.0001	0.0110	0.0001
P x Al	0.9800	0.1900	0.9600
P x cultivar	0.9800	0.2200	0.8000
AlxCultivar	0.9300	0.4200	0.4500
P x Al x cultivar	0.9800	0.7500	0.9100
Rep	0.0001	0.0001	0.0001

*. Means are followed by standard errors of mean in parentheses.

There were no significant differences in oxalate exudation between treatments of different P levels for both cultivars, although there was a significant difference due to the presence of Al. Increased Al in solution significantly increased exudation of oxalate (Table 3.12). It is reported that some plant species excrete organic acids in response to P deficiency (Gardner et al., 1983; Lipton et al., 1987). Therefore, it is possible that Al could cause P deficiency due to formation of Al-phosphate precipitates, which in turn could induce the release of organic acids. In this experiment, at either Al level, either P-free or low external P solutions did not elicit greater exudation of oxalate; neither did high external P prevent oxalate exudation in the two taro cultivars. On the other hand, addition of Al did stimulate exudation of oxalate (Table 3.12). A similar result was found in Al-tolerant wheat (Delhaize et al., 1993), in which low external P failed to stimulate malic acid excretion. If P deficiency did occur during a one week growth period and did play a role in inducing oxalate release in taro, then it would be expected that more oxalate be excreted from taro roots under P free and low P conditions compared to the high P level. The data shows that oxalate exudation in taro was specifically induced by Al stress rather than P deficiency.

Table 3.12. Effect of phosphorus levels on the exudation of oxalate in taro.

Cultivar	Al(μM)	P (μM) 0	0.3	100
		Oxalate in solution (μM)		
Bun-long	0	10.97 (0.7)*	11.77 (1.2)	11.23 (1.3)
Bun-long	900	72.07 (13.7)	95.06 (9.8)	69.44 (10.5)
Lehua maoli	0	9.54 (2.9)	8.83 (2.6)	11.34 (0.9)
Lehua maoli	900	56.82 (19.3)	63.14 (34.3)	46.11 (12.5)
		Oxalate(μg/g root fresh weight)		
Bun-long	0	48.2 (9.9)	64.2 (12.7)	59.6 (14.0)
Bun-long	900	535.9 (111.3)	693.7 (148.8)	523.6 (96.6)
Lehua maoli	0	32.1 (16.4)	19.8 (8.7)	30.7 (6.7)
Lehua maoli	900	188.2 (25.1)	284.2 (97.8)	284.2 (123.7)

Analysis of Variance

Source	Pr>F
Phosphorus (P)	0.4200
Al	0.0001
Cultivar	0.0001
P x Al	0.4300
P x Cultivar	0.6400
Al x Cultivar	0.0005
P x Al x Cultivar	0.7100
Rep	0.0150

*. Means are followed by standard errors of mean in parentheses.

Conclusions

One proposed mechanism of Al tolerance in plants is the release of Al-chelating compounds, such as organic acids, into the rhizosphere. Results of this study indicate that the exudation of oxalate from taro roots exposed to Al plays an important role in tolerance of Al for taro. Oxalate has been reported previously to be a strong detoxifier of Al. The two taro cultivars exuded significantly more oxalate

under Al stress compared to nonstressed conditions. Oxalate exuded from taro roots increased as Al concentration in solution was increased. Oxalate added to nutrient solution containing Al was shown to ameliorate Al toxicity as measured by root elongation. Finally, the exudation of oxalate from taro roots was solely a response to Al stress rather than low P.

Chapter 4

Differential Response of Seven Taro Cultivars to Aluminum Toxicity

Abstract

Aluminum (Al) toxicity is one of the most important inhibiting factors for plant growth in acid soils. To determine the existence of differential Al-tolerance within the taro [*Colocasia esculenta* (L.) Schott] germplasm, tissue-cultured plantlets of seven taro cultivars, i.e., Bun long, Filipino shortstem, Lehua maoli, Mana lauloa, Maui lehua, Niue, and Zuiki, were grown in hydroponic solution at two levels of Al (0 and 890μM Al) for 36 days. Fresh and dry weights of leaf blades, petioles, and roots, as well as root lengths, leaf areas, and photosynthesis rates were significantly decreased in the presence of Al. The presence of Al in solution significantly increased Al concentrations in leaf blades. However, cultivar differences in Al concentrations in leaf blades were not significant. Single degree of freedom contrasts indicated significant cultivar differences in response to Al among certain cultivars as measured by root length and root fresh and dry weights. Cultivars 'Niue' and 'Zuiki' were found to be more tolerant than 'Bun long'. However, no significant cultivar differences were detected between 'Bun long' and the other four cultivars tested.

Materials and Methods

Seven taro cultivars ('Bun long', 'Filipino shortstem', 'Lehua maoli', 'Mana lauloa', 'Maui lehua', 'Niue', and 'Zuiki') were grown in aerated nutrient solution culture at two initial levels of Al (0 and 890μM). The experiment followed a randomized complete block design, with four replicates of a factorial combination of two Al levels and seven taro cultivars.

The basal nutrient solution was a modified Steinberg solution (Miyasaka and Webster, 1992). The macronutrient concentrations were, in mM: NH_4-N, 0.3; NO_3-N, 2.7; P, 0.1; K, 1.2; Ca, 1.0; Mg, 0.4; and S, 0.7. The micronutrients were, in μM: Mn, 2; B, 6; Zn, 1; Cu, 0.5; Mo, 0.1; Fe (as FeEDDHA), 10. Solution pH was adjusted initially to 4.0 with dilute HCl (0.1N). Solution samples were taken twice a week to monitor pH change. Nutrient solutions were replaced when pH exceeded 4.5, or when solution levels dropped below 70% of original volume.

Taro plants were grown from tissue-cultured plantlets. In preparation of experimental plant materials, tissue-cultured taro plantlets were planted into a nursery medium and placed under shade cloth for two weeks, then grown in the greenhouse for two weeks. Osmocote(N:P:K=13.5:13.5:13.5) was used to fertilize the plantlets, with 1.46 g per plant. Leaf blades and roots were removed from taro plantlets, initial fresh weight of plantlets was determined, and uniform materials were selected within replicates and within cultivars. Plantlets were then placed on plastic grills ("egg-crate" light diffusers) which were suspended above 10-L of aerated basal nutrient solutions containing 0-Al and allowed to grow for three weeks.

Treatments were imposed on December 19, 1996. Plants were grown in a greenhouse from December, 1996 to January, 1997 for 36 days. The average maximum temperature during the experiment was 41°C, while the average minimum temperature was 16°C. One week before harvesting, leaf chlorophyll content was determined, using a Minolta Chlorophyll Meter (SPAD-502, Spectrum Technologies, Inc. Plainfield, IL), and photosynthesis rate was measured using a portable photosynthesis system (model LI-6200, LI-COR, Inc. Lincoln, NB). When visible growth differences due to treatments were observed, plants were harvested and separated into leaf blades, petioles, roots, and corms. These plant parts were then rinsed successively three times in deionized water, blotted dry, and fresh weights determined. Dry weights were determined after oven-drying at 75°C or freeze-drying. Aluminum concentrations in leaf blades were analyzed. Leaf areas and root length were determined using a digital image analysis system (Decagon Devices, Pullman, WA).

Analysis of variance (ANOVA) and single degree of freedom contrasts were calculated, using SAS programs (Statistical Analysis Systems Institute, 1982). Relative tolerance to Al-toxicity among taro cultivars was determined by single degree of freedom contrasts which compared the slopes of growth parameters between the two Al levels. A probability level of 5% or less was considered to be statistically significant.

Results and Discussion

Root length. Increased Al significantly decreased root length across all cultivars (Fig. 4.1; P=0.0001). Cultivar differences were also significant (Fig. 4.1; P=0.0001). Although 'Lehua maoli' was previously reported to be more tolerant of Al than 'Bun long' (Miyasaka and Webster, 1992; Calisay, 1996), root growth inhibition for 'Lehua maoli' was greater than for 'Bun long' as found in this experiment (Table 4.1). Hence, 'Bun long' was used as a contrast with which other cultivars were compared. Single degree of freedom contrasts showed that the slopes of decreased root length in response to increased Al were significantly different for 'Niue' and 'Zuiki' from that of 'Bun long' (Fig. 4.1; Table 4.2a), with 'Bun long' appearing to be more sensitive to excess Al than the two cultivars. A comparison of root length between cultivars at 890 μM Al using single degree of freedom contrasts indicated that cultivar differences were not significant (Table 4.2b). This suggests that the greater sensitivity of 'Bun long' compared to 'Zuiki' was due to its greater root length at 0-Al rather than greater growth at 890 μM Al. No significant cultivar differences in the slopes of decreased root length in response to increased Al were found between 'Bun long' and the other four cultivars.

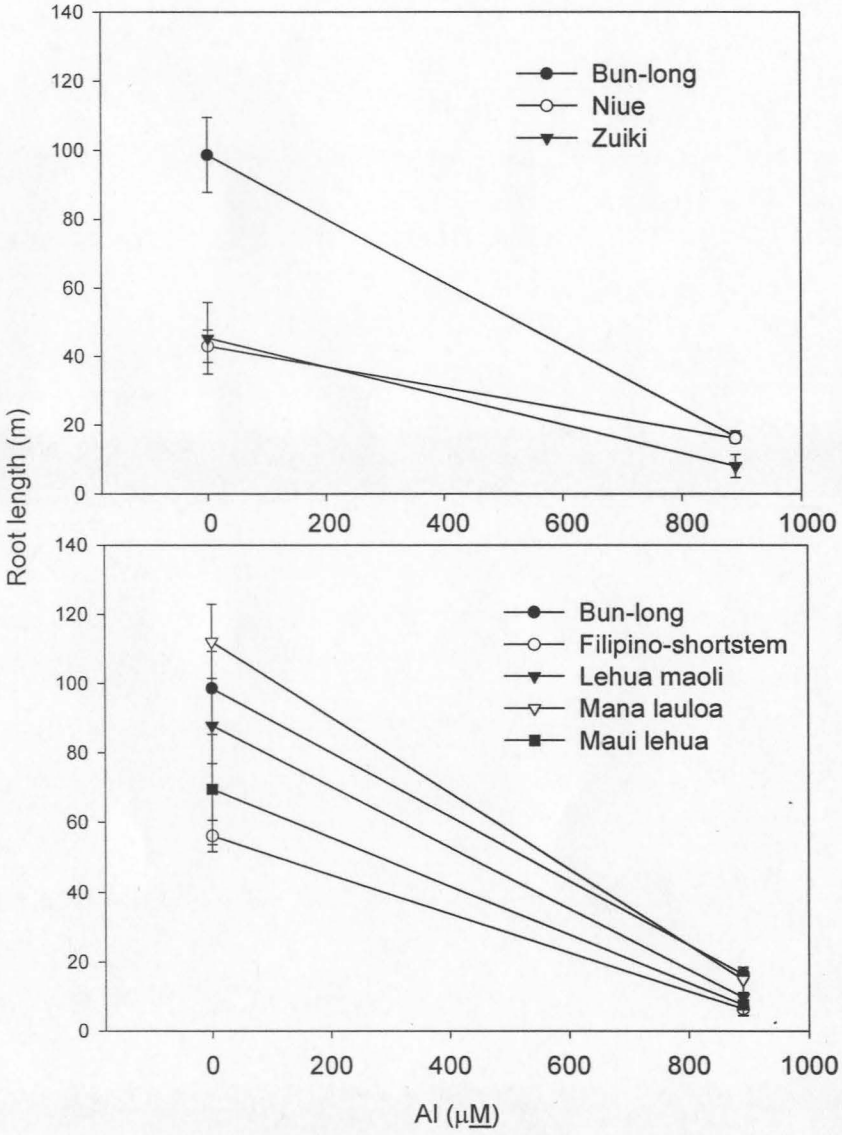


Fig 4.1. Root length of taro cultivars in the absence or presence of 890 μM Al.

Table 4.1. Root growth inhibition of seven taro cultivars under Al stress (root growth inhibition(%)=[1-(root length in Al/root length without Al)]x100).

Cultivar	Root growth inhibition (%)
Bun long	83
Filipino-shortstem	88
Lehua maoli	89
Mana lauloa	87
Maui lehua	89
Niue	63
Zuiki	82

Table 4.2a. Single degree of freedom contrasts for slope of decrease in root length or root fresh and dry weights with increasing Al levels:

	Pr > |T|		
Cultivars	Root length	Root fresh weight	Root dry weight
BL vs LM intxn	0.8100	0.2100	0.3300
BL vs FS intxn	0.0590	0.0230	0.0470
BL vs MLa intxn	0.3500	0.7200	0.7800
BL vs N intxn	0.0014	0.0060	0.0170
BL vs Z intxn	0.0081	0.0140	0.0063
BL vs MLe intxn	0.2200	0.0440	0.1100

Table 4.2b. Single degree of freedom contrasts for root length at 890 μM Al:

	Pr > |T|
Cultivars	Root length
BL vs LM intxn	0.5700
BL vs FS intxn	0.4000
BL vs MLa intxn	0.8900
BL vs N intxn	0.9600
BL vs Z intxn	0.4700
BL vs MLe intxn	0.4500

Root fresh and dry weight. Fresh and dry weights of roots across all cultivars were significantly depressed with increased Al (Figs. 4.2 and 4.3; P=0.0001 and P=0.0001, respectively). There were also significant cultivar differences (Figs. 4.2 and 4.3; P=0.0005 and P=0.0049, respectively). Single degree of freedom contrasts showed that the slopes of reduced root fresh weight with increased Al were significantly lower for 'Niue', 'Zuiki', 'Filipino shortstem', and 'Maui lehua' from that of 'Bun long' (Table 4.2a). As for root dry weight, significant differences were found between 'Bun long' and 'Zuiki', 'Niue', or 'Filipino shortstem' (Table 4.2a). 'Bun long' appeared to be more sensitive to Al-toxicity compared with 'Niue', 'Zuiki', and 'Filipino shortstem'.

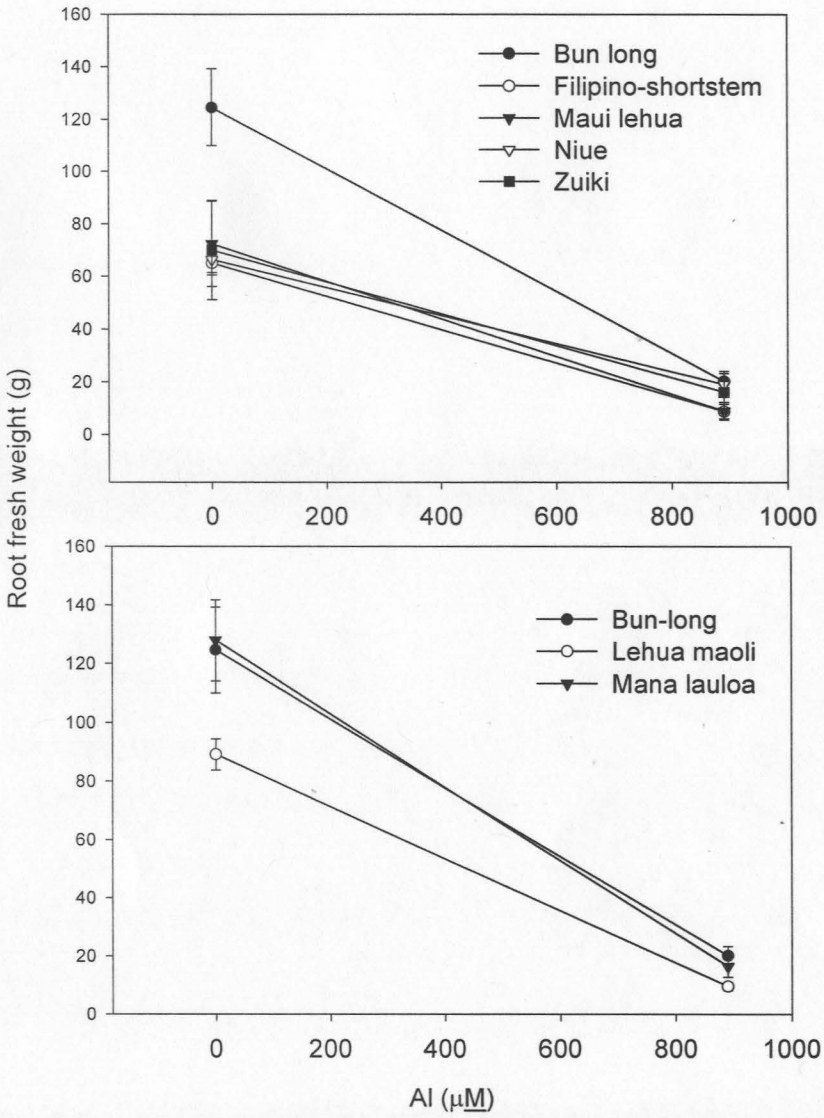


Fig 4.2. Root fresh weight of taro cultivars in the absence or presence of 890 μM Al.

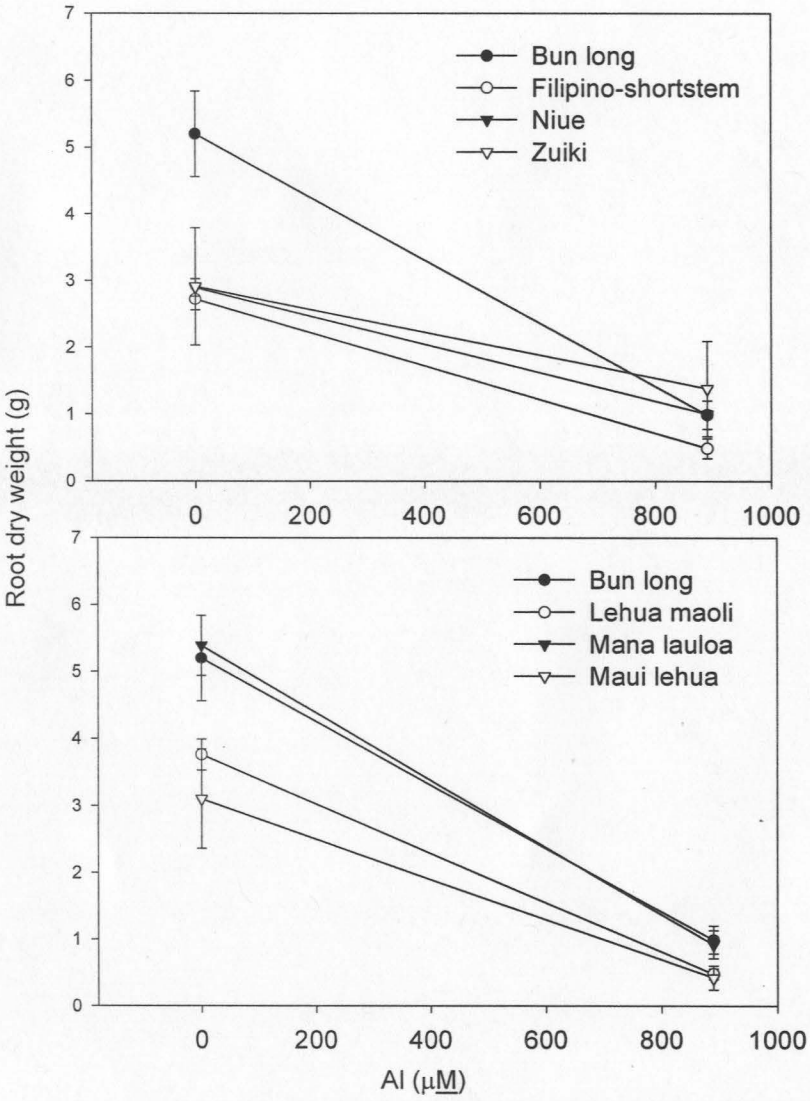


Fig 4.3. Root dry weight of taro cultivars in the absence or presence of 890 μM Al.

Other growth parameters. Increased Al significantly decreased fresh and dry weights of leaf blades and petioles, leaf area, and photosynthesis rate (Tables 4.3 and 4.4).

Table 4.3. Fresh and dry weights of leaf blades and petioles of seven taro cultivars at two Al levels.

		Leaf blade(g)		Petiole(g)	
Cultivar	Al (μM)	fresh	dry	fresh	dry
Bun long	0	57.67 (6.18)*	7.53 (0.86)	132.65 (14.25)	8.08 (1.1)
Bun long	890	10.08 (1.77)	1.11 (0.21)	24.03 (4.80)	1.89 (0.44)
Filipino-shortstem	0	48.97 (2.16)	6.27 (0.39)	116.13 (7.37)	6.37 (0.52)
Filipino-shortstem	890	7.09 (2.34)	0.86 (0.29)	14.81 (4.78)	0.88 (0.29)
Lehua maoli	0	56.33 (6.33)	7.17 (0.74)	110.13 (7.27)	6.69 (0.70)
Lehua maoli	890	5.78 (1.07)	0.56 (0.15)	16.65 (2.25)	0.88 (0.13)
Mana lauloa	0	67.91 (2.60)	8.79 (0.26)	181.49 (8.16)	11.30 (0.80)
Mana lauloa	890	11.56 (2.61)	1.22 (0.31)	23.51 (5.72)	1.40 (0.35)
Maui lehua	0	32.72 (13.22)	6.45 (1.37)	135.27 (28.09)	7.44 (1.80)
Maui lehua	890	6.49 (2.78)	0.72 (0.40)	22.62 (10.7)	1.37 (0.64)
Niue	0	51.11 (2.70)	7.23 (0.40)	147.00 (11.80)	9.32 (0.77)
Niue	890	12.89 (0.94)	1.54 (0.11)	37.04 (2.60)	2.34 (0.23)
Zuiki	0	55.48 (11.78)	5.84 (1.33)	149.02 (31.99)	7.22 (1.96)
Zuiki	890	8.03 (3.87)	0.79 (0.37)	25.50 (10.3)	1.82 (0.76)
ANOVA: P>F					
Cultivar		0.0504	0.1129	0.0584	0.0305
Al		0.0001	0.0001	0.0001	0.0001
Cultivar*Al		0.2206	0.4664	0.2833	0.1700

*Means are followed by standard errors of mean.

Table 4.4. Leaf area and photosynthetic rate of seven taro cultivars at two Al levels.

Cultivar	Al(μM)	Leaf area (cm^2)	Photosynthetic rate ($\mu mol\ m^{-2}\ s^{-1}$)
Bun long	0	2259 (202)*	13.37 (0.53)
Bun long	890	410 (83)	11.53 (0.81)
Filipino-shortstem	0	1729 (84)	14.90 (0.89)
Filipino-shortstem	890	278 (92)	10.00 (1.57)
Lehua maoli	0	1873 (182)	13.62 (2.19)
Lehua maoli	890	217 (31)	10.13 (1.00)
Mana lauloa	0	2657 (73)	13.06 (1.04)
Mana lauloa	890	507 (115)	10.27 (0.75)
Maui lehua	0	1874 (412)	12.79 (1.69)
Maui lehua	890	267 (124)	11.28 (0.40)
Niue	0	2004 (124)	12.13 (0.93)
Niue	890	531 (59)	10.46 (0.83)
Zuiki	0	2132 (416)	10.00 (1.13)
Zuiki	890	388 (188)	8.57 (0.10)
ANOVA: P>F			
Cultivar		0.0446	0.0175
Al		0.0001	0.0001
Cultivar*Al		0.5321	0.2892

*Means are followed by standard errors of mean.

Aluminum concentrations in leaf blades. The presence of Al in solution significantly increased Al concentrations in leaf blades (Table 4.5). However, no significant cultivar differences were found (Table 4.5). Leaf Al content followed a similar trend (Table 4.5). 'Bun long' had a lower content of Al in leaf blades at 890 μM Al, compared to 'Niue' and 'Zuiki', although the differences were not statistically significant (Table 4.5). In addition, 'Bun long' did not have a greater increase in Al concentration in leaf blades than 'Lehua maoli' in the presence of Al, as reported by Miyasaka et al. (1993b) and Calisay (1996).

Table 4.5.Leaf Al concentration and content of seven taro cultivars at two Al levels.

Cultivar	Al(μM)	Leaf Al concentration (μg/g leaf dry weight)	Leaf Al content (μg)
Bun long	0	3.25 (0.25)*	24.98 (4.72)
Bun long	890	22.00 (4.16)	23.73 (6.10)
Filipino-shortstem	0	3.00 (0)	18.80 (1.17)
Filipino-shortstem	890	36.67 (6.36)	41.33 (6.83)
Lehua maoli	0	3.00 (0)	21.50 (2.23)
Lehua maoli	890	21.75 (1.84)	12.97 (4.36)
Mana lauloa	0	3.00 (0)	26.38 (0.78)
Mana lauloa	890	32.75 (10.84)	31.00 (4.83)
Maui lehua	0	3.00 (0)	19.37 (4.11)
Maui lehua	890	24.67 (2.40)	23.63 (12.49)
Niue	0	3.00 (0)	21.70 (1.21)
Niue	890	54.50 (8.14)	82.15 (9.17)
Zuiki	0	3.00 (0)	17.52 (3.98)
Zuiki	890	34.50 (16.84)	44.62 (35.74)
ANOVA: P>F			
Cultivar		0.1600	0.1200
Al		0.0001	0.0230
Cultivar*Al		0.1600	0.0890

*Means are followed by standard errors of mean.

The sensitivity of 'Bun long' to Al-toxicity found in this experiment supports the result by Miyasaka et al. (1992) and Calisay (1996). In contrast, the tolerance of 'Lehua maoli' to excess Al and the variability between 'Lehua maoli' and 'Bun long' in response to Al was not detected. In earlier research, Miyasaka and

Webster (1992) and Calisay (1996) found that 'Lehua maoli' was more tolerant compared with 'Bun long', using 'hulis', which are vegetative propagating materials composed of approximately 20 cm of lower petioles and 0.5 cm of upper corm. In this experiment, tissue-cultured plantlets were used. Since tissue-cultured plantlets received growth hormones in culture, it is speculated that the response of taro to Al-toxicity might be associated with the possible effect of growth regulators. Also, the size of initial planting materials might play a role.

Conclusions

Increased Al in solution from 0 to 890 uM significantly depressed fresh and dry weights of taro leaves, petioles, and roots, as well as root length, leaf area, and photosynthesis rate. Leaf Al concentrations were significantly increased in the presence of Al. Based on root length and root fresh and dry weights, cultivar 'Bun long' appeared to be more sensitive than cultivars 'Niue' and 'Zuiki'; whereas cultivar 'Lehua maoli' did not show greater tolerance to Al-toxicity compared with 'Bun long'.

References

Akeson, M. A., D. N. Munns, R. G. Burau. 1989. Adsorption of Al^{3+} to phosphatidylcholine vesicles. Biochim. Biophys. Acta. 986: 33-40.

Barber, D. A. and K. B. Gunn. 1974. The effect of mechanical forces on the exudation of organic substances by the roots of cereal plants grown under sterile conditions. New Phytol. 73: 39-45.

Bartlett, R. J. and Riego, D. C. 1972. Effects of chelation on toxicity of aluminum. Plant and Soil 37: 419-423.

Basu, U., Godbold, D., Taylor, G. J. 1994a. Aluminum resistance in *Triticum aestivum* associated with enhanced exudation of malate. Journal of Plant Physiology 144: 747-753.

Basu, U., A. Basu, G. J. Taylor. 1994b. Differential exudation of polypeptides by roots of aluminum-resistant and aluminum-sensitive cultivars of *Triticum aestivum L.* in response to aluminum stress. Plant Physiol 106: 151-158.

Bennet, R. J., Breen, C. M., Fey, M. V. 1987. The effects of aluminum on root cap function and root development in *Zea mays L.* Environ. Expt. Bot. 27: 91-104.

Black, C. A. 1957. Soil-Plant Relationships. John Wiley & Sons, New York. 140.

Blamey, F. P. C., Edwards, D. G. and Asher, C. J. 1983. Effects of aluminum, OH:Al and P:Al molar ratios, and ionic strength on soybean root elongation in solution culture. Soil Science 136: 197-207.

Blamey, E. P. C., D. C. Edmeades, D. M. Wheeler. 1990. Role of root-exchange capacity in differential aluminum tolerance of Lotus species. J. Plant Nutr. 13(6): 729-744.

Blamey, F. P. C., Wheller, D. M. and Christie, R.A. 1990. Independence of differential aluminum tolerance in lotus on changes in rhizosphere pH or excretion of organic ligands. Journal of Plant Nutrition 13: 713-728.

Borchert, R. 1990. Ca^{2+} as developmental signal in the formation of Ca-oxalate crystal spacing patterns during leaf development in *Carya ovata*. Planta 165: 339-347.

Calisay, M. G. 1996. Differential response of taro (*Colocasia esculenta L.*) cultivars to aluminum toxicity. Dissertation of doctor of philosophy. University of Hawaii, Honolulu, HI.

Cambraia, J., F. R. Galvani, M. M. Estevao and R. Sant Anna. 1983. Effects of aluminum on organic acid, sugar and amino acid composition of the root system of sorghum (*Sorghum bicolor L.* Moench). Journal of Plant Nutrition 6(4): 313-322.

Cameron, R. C., Ritchie, G. S. P. and Robson, A. D. 1986. The relative toxicities of inorganic aluminum complexes to barley (*Hordeum vulgare L.*). Soil Science Society of America Journal 50: 1231-1236.

Clarkson, D. T. 1981. Nutrient interception and transport by root systems. pp. 307-330. In: C. B. Johnson (ed.), Physiological Processes Limiting Plant Productivity. Butterworths, London.

Dambroth, M. and N. E. Bassam. 1982. Low input varieties: definition, ecological requirements and selection. pp. 325-336. In: M. R. Saric (ed.). Genetic Specificity of Mineral Nutrition of Plants. Serb. Acad. Sci. Art. Scientific Assemblies XII. Dept. Natl. Math. Sci. No.3, Belgrade, Yugoslavia.

de la Pena, R. S. 1970. The edible aroids in the Asian-Pacific area. Proc. 2nd Int. Symp. on Trop. Root and Tuber Crops (1970), vol. 1: 136-140.

Delhaize, E., Ryan. P. R., Randall, P. J. 1993. Aluminum tolerance in wheat (*Triticum aestivum L.*) II. Aluminum-stimulated excretion of malic acid from root apices. Plant Physiol 103: 695-702.

Delhaize, E., P. R. Ryan. 1995. Aluminum toxicity and tolerance in plants. Plant Physiol. 107: 315-321.

Esau, K. 1965. Plant Anatomy. 2nd ed. Wiley, New York.

Fahn, A. 1982. Plant Anatomy. 3rd ed. pp.22-23. Pergamon Press Ltd., Oxford, England.

Foy, C. D. 1984. Physiological effects of hydrogen, aluminum and manganese toxicities in acid soil. In: Soil Acidity and Liming, pp. 57-97. Fred Adams (ed.). 2nd Edition. Agronomy Monograph No.12. American Society of Agronomy, Madison, WI.

Foy, C. D. 1988. Plant adaptation to acid, aluminum-toxic soils. Commun. Soil Sci. Plant Anal 19: 959-987.

Foy, C. D., Fleming, A. L., Burns, G. R., Armiger W. H. 1967. Characterization of differential aluminum tolerance among varieties of wheat and barley. Proc Soil Sci Soc Am 31: 513-521.

Foy, C. D., Chaney, R. L. and White, M. C. 1978. The physiology of metal toxicity in plants. Ann. Rev. Plant Physiol 29: 511-566.

Foy, C. D., E. D. Lee, S. B. Wilding. 1987. Differential aluminum tolerances of two barley cultivars related to organic acids in their roots. Journal of Plant Nutrition 10: 1089-1101.

Foy, C. D., E. H. Lee, C. A. Coradetti, and G. J. Taylor. 1990. Organic acids related to differential aluminum tolerance in wheat (*Triticum aestivum*) cultivars. In: Plant Nutrition - Physiology and Applications, pp. 381-389 (eds.) L. van Beusichem. Kluwer Academic Publ., Dordrecht, Netherlands.

Franceschi, V. R., H. T. Horner, Jr. 1980. Calcium oxalate crystals in plants. The Botanical Review 46: 361-427.

Franceschi, V. R. 1987. Oxalic acid metabolism and calcium oxalate formation in *Lemna minor L.* Plant, Cell and Environment 10: 397-406.

Franceschi, V. R. 1989. Calcium oxalate formation is a rapid and reversible process in *Lemna minor L.* Protoplasma 148: 130-137.

Gardner, W. K., D. A. Barber, D. G. Parberg. 1983. The acquisition of phosphorus by *Lupinus albus*. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. Plant Soil 70: 107-124.

Gueguen, M. F. 1908. Enations hypophylless du *Colocasia esculenta* Schott. Bull. Soc. Bot. (Fr.) 55 (Ser.4, Vol. 8): 26-32.

Hall, J. L., T. J. Flowers, R. M. Roberts. 1974a. The Golgi body. pp. 373-396. In: Plant cell structure and metabolism. Longman Group Limited. London.

Hall, J. L., T. J. Flowers, R. M. Roberts. 1974b. Cell membranes. pp. 121-132. The nucleus. pp. 133-157. In: Plant cell structure and metabolism. Longman Group Limited. London.

Haug, A. 1984. Molecular aspects of aluminum toxicity. C. R. C. Crit. Rev. Plant Sci. 1: 345-373.

Hue, N. V., Craddock, G. R., and Adams, F. 1986. Effect of organic acids on aluminum toxicity in subsoils. Soil Science Society of America Journal 50: 28-34.

Jayman, T. C. Z. and S. Sivasubramaniam. 1975. Release of bound iron and aluminum from soils by the root exudates of tea (*Camellia sinensis*) plants. J. Sci. Food Agric 26: 1895-1898.

Jones, L. H. 1961. Aluminum uptake and toxicity in plants. Plant and Soil 13: 297-310.

Kamprath, E. J. 1984. Crop responses to lime on soils in the tropics. pp. 349-368. In: F. Adams (ed.). Soil Acidity and Liming (Agronomy 12, 2nd Edition), Amer. Soc. Agron., Madison, Wisconsin.

Kasim, F., Wassom, C. E. 1990. Genotypic response of corn to aluminum stress. I. Seedling tests for measuring aluminum tolerance in nutrient solutions. Indonesian J. Crop Sci. 5: 41-51.

Kasim, F., Haag, W. L., Wassom, C. E. 1990. Genotypic response of corn to aluminum stress. II. Field performance of corn varieties in acid soils and its relationship with performance at seedling stage. Indonesian J. Crop Sci. 5: 53-65.

Keolanui, R., S. Sanxter, J. R. Hollyer. 1993. Handbook for commercial-scale taro (*Colocasia esculenta*) tissue culture in Hawaii. Research extension series 145. Hawaii Institute of Tropical Agriculture and Human Resources. Honolulu, Hawaii.

Kerven, G. L., Asher, C. J., Edwards, D. G., Ostatek-Boczynski, Z. 1991. Sterile solution culture techniques for aluminum studies involving organic acids. Journal of Plant Nutrition 14: 975-985.

Kerven, G. L., P. L. Larsen, L. C. Bell, and D. G. Edwards. 1995. Quantitative ^{27}Al NMR spectroscopic studies of Al(III) complexes with organic acid ligands and their comparison with GEOCHEM predicted values. Plant and Soil 171: 35-39.

Kinraide, T.B. 1988. Proton extrusion by wheat root exhibiting severe aluminum toxicity symptoms. Plant Physiol 88: 418-423.

Kinraide, T. B. 1991. Identity of the rhizotoxic aluminum species. Plant Soil 134: 167-178.

Kochian, L. V. 1995. Cellular mechanisms of aluminum toxicity and resistance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol 46: 237-260.

Langworthy, C. F., H. J. Deuel. 1922. Digestibility of raw rice, arrow-root, canna, cassava, taro, tree-fern, and potato starches. J. Biol. Chem. 52: 251-261.

Larsen, N. P., M. R. Jones, G. P. Pritchard. 1934. Dental decay as an indicator of a dietary fault. Amer. J. Dis. Children 48: 1228-1233.

Lazof, D. B., J. G. Goldsmith, T. W. Rufty, and R. W. Linton. 1996. The early entry of Al into cells of intact soybean roots. Plant Physiol. 112: 1289-1300.

Lee, E. H., C. D. Foy. 1986. Aluminum tolerances of two snapbeans related to organic acid content evaluated by high-performance liquid chromatography. Journal of Plant Nutrition 9: 1481-1498.

Libert, B, V. R. Franceschi. 1987. Oxalate in crop plants. J. Agric. Food Chem 35: 926-938.

Lipton, D. S., R. W. Blanchar, D. G. Blevins. 1987. Citrate, malate, and succinate concentration in exudates from P-sufficient and P-stressed *Medicago sativa* L. seedlings. Plant Physiol 85: 315-317.

Little, R. 1988. Plant soil interactions at low pH: Problem solving - The genetic approach. Commun. in Soil Sci. Plant Anal. 19: 1239-1257.

Marschner, H. 1991. Mechanisms of adaptation of plants to acid soils. Plant Soil 134: 1-20.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. Academic Press, San Diego, CA.

Matsumoto, H., Hirasawa, E., Morimura, S., Takahashi, E. 1976. Localization of aluminum in tea leaves. Plant Cell Physiol. 17: 627-631.

Matsumoto, H. 1988. Changes of the structure of pea chromatin by aluminum. Plant Cell Physiol. 29(2): 281-287.

Miyasaka S.C., J. G. Buta, R. K. Howell, C. D. Foy. 1991. Mechanism of Aluminum tolerance in snapbeans. Plant Physiol 96: 737-743.

Miyasaka, S. C., and C. M. Webster. 1992. Variability in taro germplasm to Al-toxicity. Proc. Plant Stress in Trop. Environ. 73-75.

Miyasaka, S. C., C. M. Webster, N. V. Hue. 1993a. Differential response of two taro cultivars to aluminum: I. Plant growth. Commun. Soil Sci. Plant Anal. 24(11&12): 1197-1211.

Miyasaka, S. C., C. M. Webster, E. N. Okazaki. 1993b. Differential response of two taro cultivars to aluminum: II. Plant mineral concentrations. Commun. Soil Sci. Plant Anal. 24(11&12): 1213-1229.

National Academy of Sciences. 1975. Underexploited tropical plants with promising economic value. U. S. Acad. of Sci. pp. 37-43.

Ownby, J. D., H. R. Popham. 1989. Citrate reverses the inhibition of wheat root growth caused by aluminum. J. Plant Physiol. 135: 588-591.

Parker, D. R., Bertsch P. M. 1992. Formation of the "Al13" tridecameric polycation under diverse synthesis conditions. Environ Sci. Technol. 26: 914-921.

Pavan, M. A., Bingham, F. T., Pratt, P. F. 1982. Toxicity of aluminum to coffee in ultisols and oxisols amended with $CaCO_3$, $MgCO_3$ and $CaSO_4.2H_2O$. Soil Science Society of America Journal 46: 1201-1207.

Payne, J. H., G. J. Ley, G. Akau. 1941. Processing and chemical investigations of taro. Hawaii Agr. Exp. Sta. Bull. 86.

Pellet, D. M., Grunes, D. L. and Kochian, L. V. 1995. Organic acid exudation as an aluminum-tolerance mechanism in maize (*Zea mays L.*). Planta 196: 788-795.

Pellet, D. M., L. A. Papernik, and L. V. Kochian. 1996. Multiple aluminum-resistance mechanisms in wheat: roles of root apical phosphate and malate exudation. Plant Physiol 112: 591-597.

Peterson, C. A. 1988. Exodermal Casparian bands: their significance for ion uptake by roots. Plant Physiol. 72: 204-208.

Potgieter, M. 1940. Taro (*Colocasia esculenta*) as a food. J. Amer. Dietet. Asso. 16: 536-540.

Ritchie, G. S. P. 1989. The chemical behaviour of aluminum, hydrogen and manganese in acid soils. In : A. O. Robson (ed) Soil acidity and plant growth. pp. 1-9. Academic Press, Sydney, Australia.

Ryan, P. R., Ditomoso, J. M., Kochian, L. V. 1993. Aluminum toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. J Exp Bot 44: 437-446.

Ryan, P. R., E. Delhaize, P. J. Randall. 1995. Malate efflux from root apices and tolerance to aluminum are highly correlated in wheat. Aust. J. Plant Physiol 22: 531-536.

Sakai, W. S., M. Hanson. 1974. Mature raphid and raphid idioblast structure in plants of the edible aroid genera Colocasia, Alocasia, and Xanthosoma. Ann. Bot. 38: 739-48.

Sakai, W. S. 1979. Aroid root crops, acridity and raphides. In: Tropical Foods: chemistry and nutrition. pp. 265-278. (ed.). G. E. Inglett and G. Charalambous, Academic Press, New York.

Sampson, M., Clarkson, D. T., Davies, D. D. 1965. DNA synthesis in aluminum-treated roots of barley. Science 148: 1476-1477.

Sasaki, K. 1963. Studies on oxalic acid metabolism in Begonia plants. Bot. Mag. 76:49-58.

Shuman, L. M., D. O. Wilson, and E. L. Ramseur. 1991. Amelioration of aluminum toxicity to sorghum seedlings by chelating agents. Journal of Plant Nutrition 14(2): 119-128.

Sjoberg, S. and Lars-Olof Ohman. 1985. Equilibrium and structural studies of silicon (IV) and aluminum (III) in aqueous solution. Part 13. A potentiometric and ^{27}Al nuclear magnetic resonance study of speciation and equilibria in the aluminum (III)-oxalic acid-hydroxide system. J. Chem. Soc. Dalton Trans. 2665-2669.

Smith, W. H. 1976. Character and significance of forest tree exudates. Ecology 57: 324-331.

Standal, B. R. 1983. Nutritive Value. IN: Taro: A Review of *Colocasia esculenta* and its Potentials, pp. 141-147 (ed.) J. K. Wang. University of Hawaii Press, Honolulu, HI.

Statistical Analysis Systems Institute. 1982. SAS User's Guide: Statistics. SAS Institute Inc., Cary, North Carolina.

Strauss, M. S., G. C. Stephens, J. Arditti. 1982. Variability in taro seedling population. In: Proc., Int. Symp. on Tropical Root and Tuber Crops, pp. 611-614

(ed.). E. H. Belen and M. Villanueva. Philippine Council for Agric. and Resources Res., Los Banos, Philippines.

Suhayda, C. G., Haug, A. 1986. Organic acids reduce aluminum toxicity in maize root membranes. Physiol. Plant 68: 189-195.

Sunell, L. A., P. L. Healey. 1978. Distribution of calcium oxalate crystals in taro (*Colocasia esculenta*). Amer. J. Bot. Misc. Publ. 156:23.

Sunell, L. A., J. Arditti. 1983. Physiology and phytochemistry. In: Taro - A review of *Colocasia esculenta* and its potentials. pp.35-55. (ed.). Wang, J. K. University of Hawaii Press, Honolulu, HI.

Tang, C. S., W. S. Sakai. 1983. Acridity of taro and related plants. In: Taro - A review of *Colocasia esculenta* and its potentials. pp.148-163. (ed.). Wang, J. K. University of Hawaii Press, Honolulu, HI.

Taylor, G. J. 1987. Exclusion of metals from the symplasm: A possible mechanism of metal tolerance in higher plants. J. Plant Nutr. 10: 1213-1222.

Taylor, G. J. 1988. The physiology of aluminum tolerance in higher plants. Commun. Soil Sci. Plant Anal. 19: 1179-1194.

Taylor, G. J. 1988. Physiology of aluminum phytotoxicity. IN: Metal ions in biological systems: aluminum and its role in biology, pp 123-164 (ed.) H. Sigel. Marcel-Dekker, NY.

Taylor, G. J. 1991. Current views of the aluminum stress response; the physiological basis of tolerance. Curr. Top. Plant Biochem. Physiol 10: 57-93.

Thomas, F, A. Masion, J. Y. Bottero, J. Rouiller, F. Genevrier, D. Boudot. 1991. Aluminum (III) speciation with acetate and oxalate. A potentiometric and ^{27}Al NMR study. Environ. Sci. Technol. 25(9): 1553-1559.

Tice, K. R., D. R. Parker, D. A. DeMason. 1992. Operationally defined apoplastic and symplastic aluminum fractions in root tips of aluminum-intoxicated wheat. Plant Physiol. 100: 309-318.

Vancura, V. and A. Hovadik. 1965. Root exudates of plants. II. Composition of root exudates of some vegetables. Plant Soil 22: 21-32.

Vierstra, R. and A. Haug. 1978. The effects of Al^{3+} on the physical properties of membrane lipids in *thermoplasma acidophilum*. Biochem. Biophys. Res. Commun. 84: 138-143.

Wagatsuma, T. 1984. Characteristics of upward translocation of aluminum in plants. Soil Sci. Plant Nutr. 30(3): 345-358.

Wagatsuma, T., Kaneko, M., Hayasaka, Y. 1987. Destruction process of plant root cells by aluminum. Soil Sci. Plant Nutr. 33: 161-175.

Wallace, S. U., Anderson, I. C. 1984. Al toxicity and DNA synthesis in wheat roots. Agronomy Journal 76: 5-8.

Wang, J. K. 1983. Introduction. IN: Taro - A Review of *Colocasia esculenta* and its Potentials. pp.3-13. (ed.) J. K. Wang. University of Hawaii Press, Honolulu, HI.

Wissemeier, A. H., Klotz, F., Horst, W. J. 1987. Aluminum induced callose synthesis in roots of soybean (*Glycine max L.*). J. Plant Physiol. 129: 487-492.

Wright, R. J., J. L. Hern, V. C. Baligar, O. L. Bennett. 1985. The effect of surface applied soil amendments on barley root growth in the acid subsoil. Comm. Soil Sci. Plant Analysis. 16: 179-192.

Wright, R. J., Baligar, V. C., Wright, S. F. 1987. Estimation of phytotoxic aluminum in soil solution using three spectrometric methods. Soil Sci. 144: 224-232.

Zhang, G., G. J. Taylor. 1989. Kinetics of aluminum uptake by excised roots of aluminum-tolerant and aluminum-sensitive cultivars of *Triticum aestivum L.* Plant Physiol. 91: 1094-1099.

Zhang, G., G. J. Taylor. 1990. Kinetics of aluminum uptake in *Triticum aestivum L.* Plant Physiol. 94: 577-584.